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VETERINARY MEDICINE

JUL[illegible] 1940

JUN 16 1940

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VOL. XXXV
No. 7

Entered as second-class matter Nov. 3, 1920, at the post office at Chicago, Ill., under the Act of March 3, 1879.

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VOL. XXXV JULY, 1940 No. 7

Published Monthly by the

VETERINARY MAGAZINE CORPORATION

7632 S. CRANDON AVE., CHICAGO, ILL., U. S. A.

$4.00 a year; per copy 50 cents; Special Issues $1.00

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Four types of hounds for fox hunting have been imported into this country since the beginning of the present century: (1) English foxhound, (2) Welsh foxhound, (3) fell hound and (4) Kerry beagle. Earlier importations have been merged into the American foxhound.

* * * *

The Michigan State College has awarded the contract for the construction of a forty thousand dollar addition to the veterinary clinic building. The new quarters will house horse and cattle stalls, an autopsy room and an operating room. Last year the main clinic building was constructed at a cost of $133,000.

* * * *

Seventy per cent of the cod-liver oil used in this country has heretofore come from Norway and other Scandinavian fisheries. This source is cut off by the present European war. The supply of botanical drugs, which comes largely from central and eastern Europe, was already much disrupted before Italy joined the belligerents, and Europe as a source of botanical drugs is now entirely cut off. These drugs include ergot, lavender flowers, orange peel, cascara, cantharides, camomile, buchu, henna, peppermint, gentian, arnica flowers, senega, anise, juniper berries, poppy, fennel, asafetida, gum arabic, myrrh and benzoin.

Although birds are generally considered but slightly susceptible to Aujeszky's disease, it has been proved transmissible to chickens, ducks, pigeons, geese, buzzards, and hawks, and Remlinger and Bailly recently showed the turkey is susceptible.

* * * *

Meat consumption in the United States during the past six months is reported to have been about 11% higher than for the corresponding period a year ago. Most of the increase was in pork, which has averaged 5% to 35% lower in price than a year ago. The consumption of lard was 15% greater and of beef 5% greater than a year ago.

* * * *

Since last December "a peculiar and hitherto unknown snout and foot disease which appears in the form of blisters" has existed in 15 counties in central and southern California and caused the State Department of Agriculture much concern. Eight veterinarians employed by the department have been engaged in locating and quarantining affected herds. Dr. U. C. Duckworth, Chief of the State Division of Animal Industry, emphatically denies that it is foot-and-mouth disease. Perhaps it is a coincidence that 15 affected counties run through one of the principal garbage feeding centers in the country—or perhaps it is natural.

Conquest of the entire world of disease-producing germs seems possible as a result of the discovery, reported to the National Academy of Sciences, of germs in garden and field soil which destroy disease germs of the gram-negative group.—*Sci. N. L.*

* * * *

After a seven-year trial at the Mt. Sinai Hospital, New York, a five-day treatment for syphilis has been released for general use. It consists of continuous intravenous injection of arsenic compounds for five days. It is believed that this treatment makes possible the eradication of the disease.

* * * *

The pain threshold in man is relatively stable, sensitivity varying not more than 8% in different persons. Variations in the reaction to pain depend upon individual experience and attitude, according to research workers at the Cornell University Medical College.

* * * *

Joliffe and associates of New York University report brilliant results in the treatment of "encephalopathic syndrome," a condition heretofore nearly always fatal, by the administration of nicotinic acid. They regard this condition, which is frequently seen in chronic alcoholics and endemic pellagrins, as a nicotinic acid deficiency upon which is often superimposed a vitamin B_1 deficiency.

* * * *

Typhus, which killed millions of people in Eastern Europe following the World War, reappeared recently in five Hungarian villages near the Polish border. Budapest health officials requested samples of two new American anti-typhus vaccines, one made in the U. S. Public Health Laboratories in Montana and the other by the Lederle Laboratories, Inc., Pearl River, N. Y. Dr. Adolph Eichhorn, of the Federal Bureau of Animal Industry, sailed at once for Budapest with 5,000 doses of each type of the new vaccine. On his return, he stated that the test was being made on 15,000 persons — 5,000 vaccinated with the Lederle vaccine, 5,000 with the U. S. Public Health vaccine and 5,000 controls.

The foxglove had been known for centuries before Withering, a medical practitioner of Shropshire, England, studied its properties and made a clinical evaluation of the plant as a heart tonic. That was in 1785. Since that time the problem of producing digitalis in more stable forms has vexed the medical profession.

* * * *

Dr. Wm. Robinson of the U. S. Department of Agriculture, who in 1935 discovered that the healing effect of maggots in a wound is due to allantoin excreted by the parasites, and later that solutions of urea have the same effect, has made the further discovery that a much simpler compound, ammonium bicarbonate, is equally efficacious in promoting wound healing.

* * * *

Sulfathiazole, a new member of the sulfanilamide family, was found by Flippin and Schwartz of the University of Pennsylvania School of Medicine to be preferable to sulfapyridine in the treatment of pneumonia. It is less toxic and apparently somewhat more effective. Of 100 cases treated with sulfapyridine, 15 died; of 100 treated with the new remedy, 12 died. However, of the 27 deaths, nine occurred within 24 hours after the patients were admitted to the hospital and should not be charged to a failure of either chemical. Subtracting these, the mortality was 9%. Before the use of sulfanilamide and related compounds the mortality from pneumonia exceeded 35% in the best hospitals, even using serum.

* * * *

In 1908, 119 medical schools required a high school education or less for matriculation; 26 schools required one year of college, and 11 schools required two years of college. At present, 11 schools require two years of college; 61 require three years of college, and five require a bachelor's degree or its equivalent for entrance into the medical curriculum. This extraordinary record of progress should be sufficient to indicate to anyone that American medical education is not in the slough of despondency, or the doldrums of lassitude, it is not undernourished, badly housed and insufficiently clothed.—*J. A. M. A.*

Eudora, Arkansas, has a compulsory anti-rabies vaccination ordinance, though from the first of the year until the end of March only 37 dogs were vaccinated.

* * * *

The *Monroe (Mich.) News* states: "Every man, woman and child in Monroe County is a potential victim of the rabid dog" because tenderhearted supervisors would not compel the residents to obey the dog laws.

* * * *

In 1939 dogs bit 8,093 persons in Detroit. In the first three months of 1940, 1200 dog bites were reported in the same city. Last year 1,330 persons received the Pasteur treatment. Health authorities estimated rabies cost the city at least $100,000 in 1939. In the neighborhood of 60,000 dogs were licensed in Detroit in 1939.

* * * *

The driver of a school bus and some of the school boys in Ohio captured a wild fox. It didn't seem so very wild and bit several of them. The next day it was dead and when the brain was examined by the State Department of Health it was discovered to have been rabid.

* * * *

The directors of the Gulf Coast Kennel Club, Beaumont, Texas, have asked the city council to pass an ordinance that will require owners to keep their dogs from contacting other animals for a period of one month, on the promise that this will eliminate 90% of the rabies in the city. The method should succeed, since Dr. Fred Green, city veterinarian, has just vaccinated 1,000 dogs in Beaumont.

* * * *

As a result of 22 cases of rabies in dogs in Dayton, Ohio in a period of three months, the authorities have ordered a city-wide quarantine for the duration of the outbreak. The quarantine provides that all dogs in the city must be kept on the owner's premises unless they are on a leash. It is stated that for humane reasons muzzling will not be required. Forty-seven quarantined counties in Southern Michigan have also ruled against the muzzle on the ground that it is inhumane. Kalamazoo, in the same state, is against it because it is ineffective.

In Jasper, Texas, the Boys' Scout troop assisted in an anti-rabies campaign by rounding up dogs and taking them to veterinarians for vaccination. The veterinarians donated their services.

* * * *

Dr. J. S. Pomeroy, health officer for Los Angeles County, California complains that there are 45 dog-licensing ordinances in the county, and none of them enforced. Unenforced rabies quarantines worry him also.

* * * *

After more than four years the rabies quarantine has been lifted from most of Westchester County, N. Y., and it is expected the remainder of the county, the towns of Courtland and Yorktown, will be freed during the coming summer. Lifting the quarantine means dogs may be at large without wearing a muzzle or being held on a leash. This sort of a quarantine seems "slow but sure"—to take a long time.

* * * *

Rabies and what to do about it has been a moot subject in Battle Creek, Mich., for many months. Nothing else has provoked so many letters to the "What People Think About It Column." The *Battle Creek News* asked two veterinarians, Drs. C. R. Blatchford and M. M. Stiles, to prepare an authoritative statement on the subject. A most commendable article resulted; advising the employment of anti-rabies vaccine in addition to the quarantine methods which have proved ineffective in Battle Creek. They state that of the more than 4,000 dogs which Battle Creek veterinarians have vaccinated, not one has developed rabies and, further, that there is no record of vaccination having failed to control an outbreak of rabies in dogs; citing many instances of its successful use in the field against rabies spread by natural means. They point out, however, that it has failed repeatedly to prevent rabies in white mice when inoculated intracerebrally in laboratory tests, and inferentially that if Battle Creek citizens want to prevent rabies in white mice, they had better not rely upon anti-rabies vaccine; however, for the control of the disease in dogs, they recommend its use in addition to quarantine and the collection of strays.

More Meat Products Made

High points in the progress of California's meat packing industry during 1939 included a 232,000 head increase in the number of animals slaughtered, expansion in the amount of meat products prepared in the state, and an increase in the number of establishments operating under state and state approved inspection. These are among the facts brought out in the annual report of Dr. A. G. Boyd, assistant administrator of the division of animal industry of the California State Department of Agriculture.

At the close of the year, according to the report, 436 establishments were operating under state or state approved meat inspection, an increase of six over 1938. Inspection was conducted in 115 cities, located in 36 counties, and was performed by 154 state inspectors. The 1940 census is expected to bring additional counties under state inspection, which is mandatory in counties of more than 27,000 population.

Total number of animals slaughtered in the state during 1939 under federal, state and state approved inspection numbered 5,790,886, apportioned as follows: Federal inspection, 1,682,244; state inspection, 3,444,-713, and state approved inspection, 663,929.

During the year, 85,828,052 lbs. of meat products were manufactured under state inspection and 100,358,385 lbs. under approved inspection. The combined increase in output of meat products over 1938 amounted to approximately 23,000,000 lbs. A total of 7,954,000 lbs. of fresh and processed meat was condemned in establishments under state and state approved inspection.

"Continued progress has been made in sanitary facilities to handle meat and products," notes the report. "During the year it has been possible to assign a supervising inspector to devote his full time to major improvements in meat plants. This service has proved of real value to the meat industry and has assisted materially in unifying construction."

A special publication, "Some Sanitary Aspects of Meat Plant Construction," was brought out in 1939. In connection with sanitation, the report said that many of the state's newer plants were incorporating the latest and most durable materials and equipment to simplify maintenance and reduce costs.

According to the report, 2,205 samples of meat, meat products and miscellaneous materials (oils and fats, cereals, curing materials, etc.) were examined during the year by the meat inspection laboratory. Additional work was conducted on the determination of dry skim milk in meat products. The laboratory has adopted a colorimetric method for determination of lactose. Of 1,435 labels for meat and meat products submitted for examination, 1,275 were approved and 160 disapproved.

Numerous visits were made by supervising inspectors to all slaughtering establishments in counties where mandatory inspection is not required by law. These visits, the report states, have resulted in great improvement in the sanitary condition of uninspected plants. The department's film on meat inspection, "From Range to Range," was shown and discussed with service clubs, women's organizations and other groups, and arrangements are being made for a revised edition in color. —*The National Provisioner* 102:12.

* * * *

Turkeys, tobacco and tomatoes are three popular items credited with being strictly American in origin. The turkey business is said to be the largest animal husbandry industry in the nation, native to America.—*L. L. Engel.*

* * * *

During the last five years, the consumption of meat and lard in the United States averaged only about 12% greater than during a similar period 25 years ago. Nevertheless, our population has increased about 40% as compared with a quarter-century ago. The United States is considerably behind other nations in this respect. From 1935 to 1939, inclusive, our per capita consumption has averaged only about 137 pounds a year, while in Argentina it was 326 pounds; in New Zealand, 321 pounds; and in Australia, 205 pounds.—*George A. Schmidt,* Chairman of the Board, Institute of American Meat Packers.

Fatalities from Encephalomyelitis Vaccination

There is much concern in some sections over death losses that have attended the second injection of encephalomyelitis vaccine during the present season. The percentage of these losses is higher than at the beginning of the 1939 season, when an occasional loss occurred. The condition is serious. State veterinarians in Illinois and Minnesota have issued warnings to the practitioners that may virtually stop vaccinating for the time being in those states.

There is no definite information available as to the cause of these losses; but it is noted that they did not occur in 1938, when the vaccine was used as fast as it could be prepared and none of it stored. At the beginning of the 1939 season some vaccine was used that had been stored for some weeks, and there were a considerable number of untoward reactions to the second injection, a few of the animals dying. Later in the season, when the fresh vaccine was being used, these violent reactions were less frequent.

Vaccination was discontinued much earlier than was expected in 1939 and a large quantity of vaccine remained unused. Some of this was retested for potency and distributed this year. It appears that the violent vaccine reactions have occurred where this old vaccine was used, although much of it has been used without any such reactions. It is thought probable, therefore, that as chick embryo vaccine ages, it may develop certain substances which are highly antigenic, and animals extremely sensitive to a foreign protein develop severe reactions and even die following the second dose.

Apparently animals that were vaccinated last year are more prone to these untoward reactions than those vaccinated for the first time this season.

As a precautionary measure until there is more definite information as to the cause of reactions, most distributors of equine encephalomyelitis vaccine have called in all of the 1939 serials and are now distributing only freshly-prepared vaccine. If in an emergency it becomes necessary to use the 1939 vaccine, only one injection should be given until the uncertainty in the matter is cleared up.

Intradermal vaccination, described elsewhere in this issue, looks as if it may solve this problem of untoward reactions by avoiding them altogether, and at the same time confer a more solid immunity by a single dose than subcutaneous vaccination.

* * * *

Trichinosis Study

A bill has been introduced in the New York legislature providing for a commission "for the purpose of studying the problem of the disease known as trichinosis and other diseases contracted by eating infected meat, and possible methods of curing them." The committee is to consist of three members appointed by the governor, three from the senate, and three from the assembly.

The commission is directed in the proposed statute to "study these problems with particular attention being given to the following possible solutions:

1. Skin tests to establish the presence of trichinae in swine.
2. Refrigeration of slaughtered swine.
3. Proper cooking of garbage used for feeding swine.
4. Elimination of garbage feeding of swine.
5. Microscopic inspection of swine [pork].
6. State inspection of meat.

The sum of $5,000 is provided for the expenses of the commission and it is directed to work in cooperation with the state health department and the state department of agriculture and markets. Since no program of research for the development of new facts can be undertaken with a sum so small as $5,000, it must be assumed that the commission if created will confine itself to compiling the information already available and presenting it to the legislature officially, perhaps with its recommendations for remedial legislation. As has been pointed out in these pages[1] public discussion of the trichinosis problem, which is essentially the problem of preventing the commercial feeding of raw garbage to swine, is fraught with danger to the swine producer, and the swine processor, and may result ultimately in injury to the pork consumer, but it is far more hazardous to suppress discussion or ignore the problem.

[1] *Vet. Med.* 35:3 (March, 1940), pp. 140-141.

A. V. M. A. Responsible But Without Authority

It is an axiom in commerce and in government, both civil and military, that it is hazardous to assume responsibility without discretionary authority; yet this is just what the American Veterinary Medical Association did at the Memphis meeting in endorsing the dog food testing program inaugurated by the Committee on Foods of the American Animal Hospital Association.

The A.V.M.A. endorsed this project by the adoption of the following resolution presented by the Chairman of the Executive Board:

BE IT RESOLVED that any dog food approved by the unanimous opinion of the Scientific Advisory Council (now consisting of George R. Cowgill, Walter C. Russell, H. H. Mitchell, B. W. Fairbanks and Agnes Fay Morgan) and subsequently approved by the Committee on Foods of the American Animal Hospital Association (all of whom are members of the American Veterinary Medical Association), as long as it shall remain approved shall be granted an insigne or statement to the following effect: "Tested and Approved as Represented on Container—American Animal Hospital Association in Cooperation with the American Veterinary Medical Association."

BE IT FURTHER RESOLVED that the American Animal Hospital Association agrees to protect, keep and save harmless the American Veterinary Medical Association from any and all claims, demands, actions, or causes of action of every kind, character, nature and description whatsoever, by reason of the right herein granted to the American Animal Hospital Association.

The American Animal Hospital Association also agrees to direct the Committee on Foods to prepare an annual report in duplicate, one copy to be forwarded to the Executive Board of the American Veterinary Medical Association as an informative report.

BE IT FURTHER RESOLVED that these resolutions shall be effective when signed by the accredited officers or committee of the respective associations, subject to termination by either association upon one year's written notice. (To be duly in force when signed by executive officers of each association.

The proposition was new to a majority of the members of the House of Representatives present, but as a result of pressure from the Executive Board and of delegates and others personally interested in the outcome, the resolution was railroaded[1] through the House after a short debate—three-fourths of the time on the floor being given to a representative of the A.A.H.A. who was not a member of the House.

Members of the House of Representatives were misled by the wording of the foregoing resolution into thinking that it was proposed that the A.V.M.A. should enter into a coöperative arrangement with the A.A.H.A., with the latter enacting the leading role, although a careful reading of the resolution does not justify that assumption.

Aside from the preposterousness of a No. 50 tail wagging a No. 5000 dog, the fact has been amply demonstrated since the meeting that no coöperative arrangement was ever contemplated and none has been carried out. The endorsement of the A.V.M.A. was sought and has been used solely in an effort to break down the sales resistance to seals of approval. This was implied but not made plain during the debate on the resolution.

Almost before the members of the A.V.M.A. reached their homes from the Memphis meeting, full page advertisements were appearing in dog magazines under the headlines "American Veterinary Medical Association endorses food testing program of the American Animal Hospital Association." Not much evidence of coöperation there. In this advertisement were listed the dog foods and seal numbers of three manufacturers, the plain implication being that these foods were endorsed by the A.V.M.A. At that time there had been no chance for the A.V.M.A. to coöperate in testing those foods, not even to be consulted about flaunting its name at the head of full-page advertisements in lay magazines as endorsing the products of certain minor dog food manufacturers.

Before the end of the year similar advertisements were appearing in the dog press containing a large copy of the seal to which

[1] In response to a request for a show of hands of those who had heard of the proposition prior to the meeting, 17 were raised. Later a number of those stated they had first heard of it on the preceding day. A plea for more time to consider the matter was argued down forcefully.

the letters A.V.M.A. were added in bold, block type, and again listing the three original seal holders and one new one. The association's dog food committee would have had to act very quickly to coöperate in testing this new food. There was no need to do so, however, only to endorse the work of others.

Manufacturers applying for the seal of approval on their dog foods are required to sign a contract providing for the payment of $950 per year to get and use the seal, for additional payments under certain contingencies, for cancellation and other matters. This contract constitutes the entire agreement between the manufacturer and the source of his seal of approval, and nowhere in it is the A.V.M.A. mentioned. It is not a coöperator in this project but an endorser—a supporter—simply an aid to sales. The words on the seal "in coöperation with the A. V. M. A." are a deliberate misstatement as now and heretofore used.

In another matter in connection with this dog food testing project, the members of the House of Representatives were misled at the Memphis meeting. They were definitely assured the A.A.H.A. would protect the A.V.M.A. against financial loss as a result of legal involvement, even to the extent of posting a "bond guaranteeing the protection of the A.V.M.A. in the event that this association is involved in legal entanglements."

Damage suits should not grow out of this project, but they may. And if they do, the A.V.M.A. will be the fall guy. The A.A.H.A. has not furnished the promised bond. The A.V.M.A. is a corporate body, and is legally and financially responsible for the acts of its representatives. Its cash and securities totaling more than $50,000 are an inviting prospect to the injured, if any there should be. It is responsible for the acts of the A.A.H.A. dog food committee but has not the slightest control over its activities.

Some readers of our discussion of this dog food testing project last month have expressed astonishment and incredulity that the A.V.M.A. would endorse the delegation of dog food testing to other than veterinarians while at the same time many of its members and several of its constituent organizations are urging upon the public the wisdom of intrusting the inspection of human food to the veterinary profession.

It's in the record, however. It may be found in the published proceedings of the Memphis meeting on pages 455 to 459 inclusive of the official journal for October, 1939, where the following occurs:

DR. BIRCH: Who makes the examinations?

DR. LACROIX: *All food testing* thus far has been done at the University of Illinois. [Italics ours.]

And again on the same page (456):

DR. LACROIX: The examination of the food or the testing of the food is done at the University of Illinois. . . .

There are some half-dozen competent veterinarians at the University of Illinois and their laboratory is above reproach, but they are not concerned in this dog food testing.

If any doubt remains, we refer the incredulous to advertisements[2] of the A.A.H.A., in which the following statement occurs:

"The seal is granted to dog foods which, after thorough testing by a scientific advisory council, are approved by the Committee on Foods of the American Animal Hospital Association."

There are no veterinary members of the scientific advisory council which does this thorough testing (see list in first paragraph of resolution).

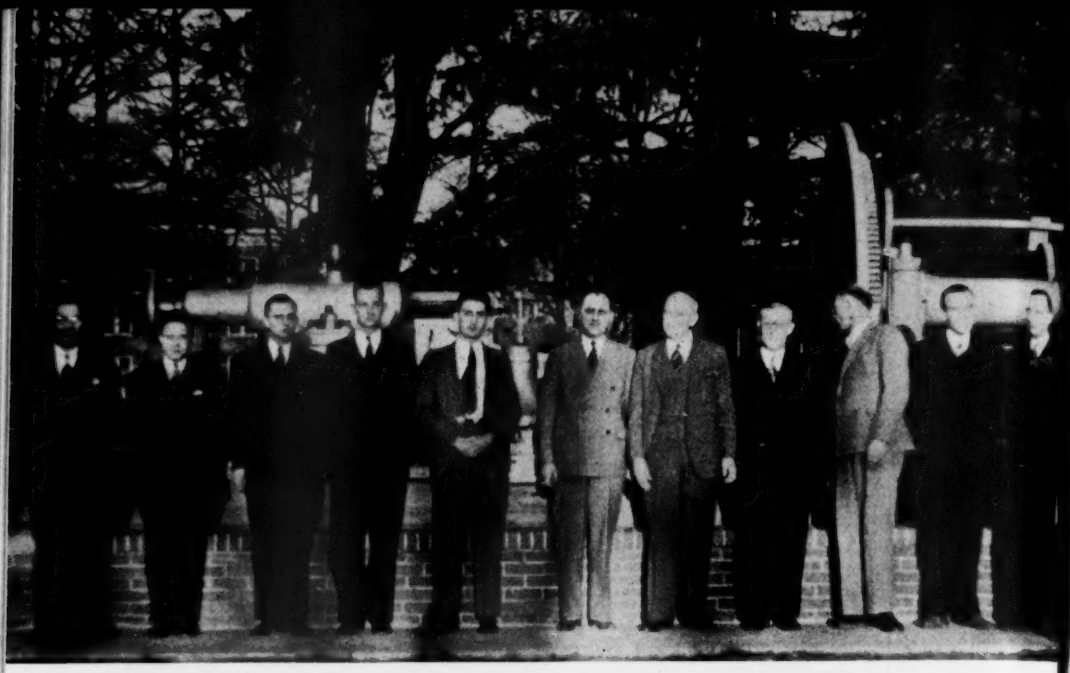
Again, we find this gem in advertisements given a circulation of many thousands among the clients of veterinarians:

"It [the recognition of nutritional adequacy as to vitamins] is not within the capacity of a layman—or even of most practicing veterinarians. Even the Committee on Foods goes for its authority to specialists. . . ."

To indicate that the Committee on Foods is different from the common, garden variety of veterinarians, the latter part of the quotation is set off by italics. The foregoing probably explains why there are no veterinarians on the scientific council.

However, persistence wins! If the searcher will follow this trail long enough he will

[2] It is not convenient to refer to all the advertisements containing these and similar statements, but readers may find copies of those from which the foregoing were taken in issues of the *American Kennel Gazette* from the time of the Memphis meeting to the present. In this connection they well repay reading *in toto*.



DELEGATES TO THE ANNUAL MEETING OF ALPHA PSI, AUBURN, ALABAMA, MARCH 21-22, 1940

Left to right: E. H. Peterson, Beta Chapter, N. Y. S. V. C.; Leo A. Wuori, Beta Chapter, N. Y. S. V. C.; John Miller, Alpha Chapter, O. S. U.; E. S. Conklin, Iota Chapter, M. S. C.; George C. Poppensiek, Epsilon Chapter, U. P.; Dr. M. C. Linneman, St. Joseph, Minn., 2nd Vice President; Dr. E. L. Booth, U. P., National Secretary; Dr. R. E. Rebrassier, O. S. U., President; Dr. F. X. Gassner, C. S. C., 1st Vice President; P. C. Olson, Kappa Chapter, W. S. C.; J. K. Bushnell, Zeta Chapter, C. S. C.; R. L. Williams, Theta Chapter, A. P. I.

The lathe visible behind the delegates is a historic object in the South. On this lathe were turned most of the cannon used by the Confederate armies in the War Between the States. It occupies a conspicuous site on the campus at the Alabama Polytechnic Institute, Auburn.

find the American Animal Hospital Association's (and now the A.V.M.A.'s) conception of the role of the veterinarian in dog food inspection. Here it is also in an advertisement of the hospital group:

"Chemical and biological tests are made in noted university laboratories. Practical tests for palatability and laxation are conducted concurrently in member hospitals."

There it is. A veterinarian can offer the food to a dog and determine its palatability in a *practical* manner by observing whether or not the animal eats it. Further, if the food is palatable, he can determine *practically* whether it causes diarrhea. So can the owner. Scientific matters are left to other than veterinarians in this amazing scheme.

Lest an injustice be done, let it be repeated that the granting of seals of approval is at all times under the *supervision* of veterinarians. The A.A.H.A. Committee on Foods collects the $950 and hands out the seal of approval. Federal meat inspection at Chicago would be equally under the *supervision* of veterinarians if it were carried out in the abattoirs by inspectors other than veterinarians but the Inspector-in-Charge at this station, the Chief of the Meat Inspection Division at Washington and the Chief of the Bureau remained as at present.

As we stated last month, this dog food testing project should be reëxamined carefully at the Washington Meeting of the A.V.M.A. with a view to developing a better and a safer plan—one that will cause less dissent among our members and one that will work. This one has not worked, and we see no prospect that it will work until a minimum standard for dog food is adopted that will effectually shut out the inferior brands and until the plan is so changed that there can be no suggestion of the sale of seals. The plan, like Caesar's wife, must be above suspicion.

Studies on the Intradermal Method of Vaccination Against Equine Encephalomyelitis

By H. W. SCHOENING, M. S. SHAHAN, O. L. OSTEEN, and L. T. GILTNER
Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture
Washington, D. C.

Introduction

As a result of occasional reports of disturbing reactions following the vaccination of horses in 1939 with two doses of equine encephalomyelitis chick embryo vaccine, attention was given to a study of these reactions and the possible development of methods of vaccination which would reduce or minimize them. Inasmuch as the intradermal method of vaccination has proved highly successful in immunization against many diseases, it was considered to have good possibilities in its application to encephalomyelitis. It was also thought that the injection of the vaccine into the skin would be less likely to result in reactions than when the vaccine was administered subcutaneously or intramuscularly. The problem, therefore, had two aspects: 1. Could a practical immunity be induced in horses by intradermal vaccination? 2. Could disturbing reactions in horses be eliminated or reduced by this method of vaccination?

The vaccines used in the following experiments, with one exception, were the regular, commercially prepared chick-embryo vaccines.

Preliminary Studies on Guinea pigs

Preliminary studies made on guinea pigs, comparing the resistance against encephalomyelitis produced by subcutaneous and intradermal injections of the vaccine in similar doses, indicated that the intradermal method of vaccination was at least as effective as, if not more effective than, the subcutaneous vaccination. Table I gives a summary of the results obtained.

Tests with Horses*

Following the favorable results of the intradermal vaccination in guinea pigs, a test of this method was next made on horses. The vaccines used in the tests herein reported were of western type, and the virus exposures, which were all intracerebral, were, of course, to the western type of virus. The vaccines themselves, except in one instance, were commercially prepared. In applying the intradermal method of vaccination to horses, the following procedure was adopted: The injection was made into the skin on the middle third of the lateral aspect of the neck. The site of injection was prepared by first shaving the skin, cleansing thoroughly with a soapy disinfectant solution, wiping the site thoroughly with dry cotton, then cleansing with alcohol, again drying with cotton, and then applying tincture of iodine. After allowing this to dry, the injection was made intradermally, using an 18- or 20-gauge, one-inch needle (needle and syringes previously sterilized). When two injections were given, the second injection was made on the opposite side of the neck from the first. No difficulty has been experienced in injecting an amount up to 1cc intradermally. The same technique was employed when vaccines were injected subcutaneously.

Experiment 1

Table II gives the results of the intradermal test on four horses. It will be noted that two of these horses received two injections intradermally August 17 and August 24, 1939, while two received a single intradermal injection of 1cc on August 24, 1939. Fifteen days after the last injection these four horses, together with two controls, were exposed to western virus by an intracerebral inoculation. Both control horses developed the disease and died, whereas of the four animals that had received the vaccine intradermally only one died, this being an animal that had received two injections.

* Of the horses used in these tests all but five had received eastern type vaccine some months previously. It has been our experience, gained from considerable experimentation, that horses vaccinated with one type of vaccine are no more resistant to exposure to the opposite type virus than normal animals.

Experiment 2

A second experiment had for its object a comparison between the immunity produced by a subcutaneous injection and by an intradermal injection of the same amount of the same vaccine. The horses were vaccinated October 18 and October 25, 1939, and were exposed by intracerebral inoculation to virulent encephalomyelitis virus 97 days after the second injection, January 30, 1940. The results of the test are given in Table III.

TABLE I. Comparative Results of Subcutaneous and Intradermal Vaccination Against Encephalomyelitis in Guinea Pigs *

Method of Vaccination	No. of Injections	No. of Guinea Pigs	Cc of Vaccine	Per Cent Survivals
Subcutaneous	2	11	0.5	90.9
Intradermal	2	11	.5	100.0
Subcutaneous	1	11	.5	72.7
Intradermal	1	12	.5	83.3
Subcutaneous	2	12	.1	91.7
Intradermal	2	12	.1	83.3
Subcutaneous	1	12	.1	33.3
Intradermal	1	12	.1	58.3
Subcutaneous	2	4	.05	75.0
Intradermal	2	6	.05	83.3
Subcutaneous	1	5	.05	0.0
Intradermal	1	6	.05	16.7
Controls	....	16	.....	0.0

* Exposure consisted of intracerbral inoculation of 0.1cc of a 1:500 dilution of guinea pig brain virus in saline. Test terminated 10 days after exposure.

It will be noted that all four of the control horses died of encephalomyelitis. The results with the smaller doses of vaccine, either in one or two injections, were "spotty" and irregular. With the 1cc dose, the results were more regular and indicated that the intradermal injection of this amount, either in one dose or in two doses, showed a higher degree of protection than the same amounts injected subcutaneously. With the subcutaneous method of vaccination, two doses of 1cc were superior to the one dose. While no difference in protection was noted between the two doses and the one dose given intradermally, it is generally accepted that in vaccination multiple injections result in the stronger immunity.

Experiment 3

A third experiment had for its object a comparison of immunity produced in horses by two 10cc doses of vaccine administered subcutaneously and two 1cc doses administered intradermally. Two lots of vaccine were used in this experiment: vaccine A (shown by tests on guinea pigs to be highly potent) and vaccine B (shown by tests on guinea pigs to be of very low potency). Two additional horses were given one injection of 0.5cc of vaccine A intradermally. The animals were vaccinated on February 19 and February 26, 1940, and were exposed April 2, 1940, 36 days after the second injection. Results of the test are given in Table IV.

TABLE II. Results from Intradermal Vaccination of Horses Against Encephalomyelitis*

Horse No.	Vaccine 8-17-39	Vaccine 8-24-39	Results
1208	1.0cc	1.0cc	Died, encephalomyelitis
1188	1.0 "	1.0 "	Remained normal
1187		1.0 "	" "
1185		1.0 "	" "
Controls			
1182			Died, encephalomyelitis
1178			" "

* All animals exposed, Sept. 8, 1939, to a 0.5cc dose of a 1:10,000 dilution of the virus (guinea pig brain), which titration was fatal to guinea pigs in a 0.1cc dose of a 1:60,000 dilution and fatal to horses in a 0.5cc dose of a 1:10,000 dilution.

All the horses exposed after treatment with vaccine A survived. Unfortunately, one of the animals vaccinated intradermally, died from an accidental injury before exposure. Of the animals vaccinated with vaccine B, which had shown low potency on the guinea pig test, all died with the excep-

TABLE III. Comparative Results of Subcutaneous and Intradermal Vaccination Against Encephalomyelitis in Horses*

Horse No.	Vaccination: Subcutaneous 10-18-39	Subcutaneous 10-25-39	Intradermal 10-18-39	Intradermal 10-25-39	Results
	cc	cc	cc	cc	
1212		0.5			Remained normal
1216		0.5			Died, enceph.
1201	0.5	0.5			" "
1202	0.5	0.5			" "
1219		1.0			" "
1220		1.0			" "
1180		1.0			" "
1157	1.0	1.0			" "
1173	1.0	1.0			" "
1175	1.0	1.0			Remained normal
1217				0.5	" "
1218				0.5	" "
1171			0.5	0.5	Died, enceph
1203			0.5	0.5	" "
1221				1.0	Remained normal
1222				1.0	Died, enceph.
1149				1.0	Remained normal
1179			1.0	1.0	Died, enceph.
1177			1.0	1.0	Remained normal
1210			1.0	1.0	" "
Controls					
1094					Died, enceph.
1117					" "
1124					" "
1159					" "

*All animals exposed, on Jan. 30, 1940, to a 0.5cc dose of a 1:10,000 dilution of the virus (guinea pig brain) which titration had shown to be fatal to guinea pigs in a 0.1cc dose of a 1:100,000 dilution and fatal to horses in a 0.5cc dose of a 1:10,000 dilution.

tion of one animal which had received two doses intradermally. Of the three control animals, two died of encephalomyelitis. The virus used in this experiment was carefully titrated in guinea pigs before the exposure of the test horses, and had been also titrated in horses. When used, it was considered to be sufficiently virulent to cause death in all normal animals. That it was quite potent is evident from the fact that three of four horses treated with the vaccine of low potency died of encephalomyelitis. However, one of the three control horses survived. It is significant that, in this test, the intradermal vaccination gave at least as good results administered in two doses of 1cc as did the two 10cc doses given subcutaneously, since all the animals treated intradermally (two treated with only 0.5cc) with the potent vaccine survived, and with the vaccine of low potency the intradermally treated animal was the only one that survived.

Study of Reactions Following Vaccination

Two tests have been made in horses to compare the effects of the same vaccine administered subcutaneously and intradermally. In these two tests vaccines were employed which had been reported to have caused severe reactions and some deaths in horses in the field this spring (1940).

In test No. 1, ten horses were given two subcutaneous injections of vaccine in the regular 10cc doses one week apart, and ten additional horses were given two injections of the same vaccine at the same interval in 1cc doses intradermally. The horses given the intradermal injection were, with one exception, worked continually. The horses in the subcutaneous vaccination group, with two exceptions, had been vaccinated with chick embryo vaccine in 1939.

Of the ten horses vaccinated subcutaneously, no appreciable reaction was noted following the first injection. Following the second injection, however, five of the eight horses which had received chick embryo vaccine in 1939 showed quite extensive swelling at the site of injection, starting at 24 hours and usually reaching a maximum in 48 to 72 hours. These reactions consisted of a hard, edematous swelling reaching a size in some animals of 12 inches wide and 18 inches long, with edema extending over the brisket and also on the opposite side of the neck. None of the animals showed any rise in temperature nor general reaction, with the exception of one which was off-feed the second day after the second injection was given, but evidenced no inconvenience other than the local reaction. These reactions gradually subsided and were considerably reduced at the end of a week. In the remaining four animals a moderate swelling, ranging from four inches long and four inches wide to practically no reaction, was noted. Of the two animals which were not vaccinated in 1939, one showed a severe reaction and the other a slight reaction. Of the ten animals injected intradermally, no reaction was noted following the first injection other than a slight edema and thickening of the skin. Following the second injection, three animals showed a slight edema approximately three inches long and three inches wide. Seven animals showed practically no reaction at the site of inoculation. The three animals first mentioned showed much less reaction than the four animals injected subcutaneously which were considered not to have shown any unusual reaction following the subcutaneous inoculation.

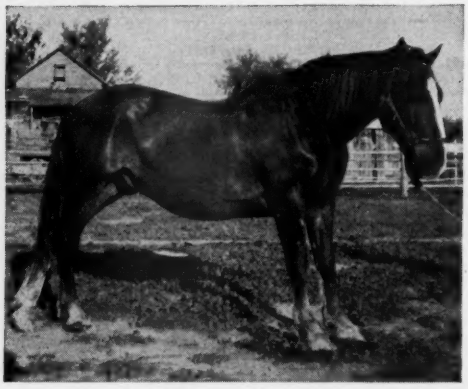
In test No. 2, ten horses were given two intramuscular injections of vaccine in the pectoral region in the regular 10cc doses,

TABLE IV. COMPARATIVE RESULTS OF SUBCUTANEOUS AND INTRADERMAL VACCINATION IN HORSES*

Horse No.	Vaccine	Vaccination: Subcutaneous 2-19 40	Subcutaneous 2-26 40	Intradermal 2-19 40	Intradermal 2-26 40	Results
		cc	cc	cc	cc	
1224	A	10	10			Remained normal
1225	A	10	10			" "
1227	A			1.0	1.0	" "
1228	A			1.0	1.0	Died, injury
1226	A				0.5	Remained normal
1190	A				0.5	" "
1229	B	10	10			Died, enceph.
1230	B	10	10			" "
1233	B			1.0	1.0	" "
1215	B			1.0	1.0	Remained normal
Controls						
1132						Died, enceph.
1232						" "
1195						Remained normal

*All animals exposed April 2, 1940, to a 0.5cc dose of 1:5,000 dilution of the virus (guinea pig brain) which was fatal to guinea pigs in a 0.1cc dose of a 1:80,000 dilution and fatal to horses in a 0.5cc dose of a 1:10,000 dilution.

one week apart. Ten additional horses were given two injections of the same vaccine at the same interval in 1cc doses intradermally on the neck following the usual procedure for intradermal injection. Of the ten horses vaccinated intramuscularly, no appreciable reaction was noted following the first injection. The second injection in six horses was made on the site of the first injection. The remaining four were given the injection on the opposite side. Following the second injection, no appreciable reaction was noted in any of the animals except one. This animal had received the second injection on the side opposite the first and had also been vaccinated in 1939. Only one other animal in this group had been vaccinated in 1939. The remaining eight animals, so far as known, had not been vaccinated prior to this test. The animal that reacted showed an extensive, edematous swelling eight inches across, protruding three inches beyond the normal line of the breast, and extending backward between the front legs. There was slight stiffness, but no rise in temperature nor other inconvenience. The swelling gradually subsided. Of the ten animals injected intradermally, no appreciable reaction was noted following either the first or second injection. Of the horses injected intradermally, eight had been vaccinated in 1939 with chick embryo vaccine and, so far as is known, two had not been vaccinated.



Typical Encephalomyelitis.—*Photo by Marsh*

Summarizing the results of the two tests: Of the 20 horses injected intradermally, 18 had been vaccinated in 1939. None of this group of 20 showed any untoward reaction. Of the 20 horses injected either subcutaneously or intramuscularly, ten had been vaccinated in 1939. Of these ten horses, six developed large swellings at the site of injection. Of the ten not vaccinated in 1939, one developed a similar swelling.

Summary

1. The intradermal injection in guinea pigs of regular, commercially prepared, chick embryo equine encephalomyelitis vaccine produced at least as good, if not a better, immunity than when the same vaccine in the same amounts was injected subcutaneously.

2. Vaccine injected intradermally in various amounts in horses produced a better immunity than the same vaccine in similar amounts when injected subcutaneously.

3. In horses the immunity produced by intradermal injection of vaccine in two 1cc doses was as good as that produced by two 10cc doses subcutaneously.

4. Vaccines reported to have caused severe, undesirable reactions in horses in the spring of 1940, when injected into 20 horses intradermally, produced very little local reaction, while the same vaccine injected subcutaneously or intramuscularly in 20 horses produced in some of the animals rather marked, extensive swellings at the site of injection. The greater number of these untoward reactions appeared in horses which had been vaccinated in 1939.

"Secondary Disease" in Montana.—*Photo by Marsh*

The Border Between Life and Death

III. The Janus-Faced Behavior of Viruses

By A. ASCOLI, New York City

LET us endeavor to establish by means of biological reactions what type of antigens correspond to the protoplasmic constituents of bacteriophages. We have several bacteriophages, a staphylophage, a coliphage, etc., and the action of each is specific. The staphylophage clears up staphylococcus cultures; the coliphage, *B. coli* cultures, and so forth. A cleared-up broth therefore contains two specific antigens, the one belonging to the dissolved microbic bodies and the other belonging to their bacteriophage.

■ The most simple tests for distinguishing the different antigenic cell content of any class of animals, plants, or microbes, are the antisera prepared by injecting suspended cells into small laboratory animals. By their neutralizing or precipitating action, it has been possible to differentiate the bacteriophages from each other and from the protoplasmic constituents of the microbes. The main method employed by Bordet and Gratia, who performed this differentiation, is essentially the same as the one by which legal medicine recognizes the source of blood samples, veterinary hygiene the adulteration of mixed meat. This is basically analogous to the proceeding used for selecting appropriate donors for blood transfusion, and even in research to determine paternity. Bordet succeeded in preparing a precipitating antiserum for the coliphage. Gratia carried on the work by crossing tests with the staphylophage antiserum, ascertaining by this elementary means the autonomous feature of every single bacteriophage.

In a cleared-up culture the presence of bacteriophage may be detected by the neutralizing or precipitating action of the appropriate antiserum. Might we not conclude from this fact that this peculiar reaction of bacteriophage which is specific for each type, is a sort of inherited trait which may indicate a biological relation to the regenerated matter as if it were an offspring?

■ The analogy of the mechanism of the suicide factor to autocatalysis of trypsinogen appears to be strongly shaken by these findings if we consider what happens when more trypsin is built up by the addition of trypsin to a pancreas extract. In this case the trypsin formed anew, for instance, by adding trypsin of swine to a pancreas extract of dog does not give rise to trypsin of swine but to a trypsin behaving, when tested by biological reactions, like that of dog pancreas extract. That signifies that in the process which occurs, only the dog pancreas extract is concerned. It seems as if a certain amount of enzyme is formed autocatalytically by its disintegration, but a new development of the trypsin of swine, which would correspond to bacteriophage, cannot be detected. The fact that by its behavior toward antisera the newly-built trypsin is linked to the dog proteins, emphasizes in this case the assumption of its origin from the latter. This actually may be an autocatalytic process, but the hypothesis that a similar mechanism occurs in bacteriophagism is weakened by the observation that, whenever the clearing up starts, a new amount of bacteriophage-like reacting material is formed. The biological tests reveal such a contrast in the course of the two processes that it becomes rather unlikely to conceive the mechanism of bacteriophagism as due to autocatalysis—at least that any support to such a view can be drawn from the analogy hinted by Bordet.

■ If one prepares appropriate antisera by injecting into a group of rabbits the juice of healthy tobacco leaves and into another group the juice of tobacco mosaic diseased leaves, one finds reagents by which it is possible to differentiate the components of the tobacco mosaic virus from the normal constituents of the tobacco plant. By means of these reagents Mrs. Chester has suc-

ceeded in showing that whenever a healthy leaf, in which previously only normal constituents were detectable, becomes diseased, the characteristic reaction of tobacco mosaic virus protein starts at once, *i.e.*, the juice which was not precipitable hitherto gives rise to a flocculation when tested with tobacco mosaic antiserum, while the flocculation occurring in the antiserum of healthy leaves becomes weaker and weaker. As in the clearing broth it was found that bacteriophage had increased, so in the diseased leaves, a new component, tobacco mosaic virus protein appeared. This substance can be detected easily by means of precipitation tests. As in the case of bacteriophage no relationship to the normal cell proteins from which it might originate autocatalytically can be found.

■ Bordet's hypothesis which assumes a relationship of bacteriophage's activity to the organic catalysts, did not succeed in explaining its enigmatic behavior. Autocatalysis would have been a plausible solution of the enigma, since enzymes are actually organic catalysts, but when all factors were taken into consideration the hypothesis broke down. Neither does d'Herelle's assumption that bacteriophage behaves itself as a living source of enzymes find any support, but it reminds us of the debate on the living nature of yeast fermentation which was not definitely closed until Buchner succeeded in squeezing a juice from the *Saccharomyces cerevisiae,* which started the enzymatic process in sugar solutions, though none of the cells had survived the high pressure of his famous "Buchner press."

Strange mystery of nature! Just when this problem, which had been vehemently debated by biologists during the second part of the past century, reached its settlement, a new and perplexing question arose due to Beijerinck's observation that the tobacco mosaic virus by reason of its small size was able to pass through earthenware candles with pores not large enough for the passage of the average of microbes. This experiment paved the way to the discovery that many infectious diseases, not only of plants but of animals and men, were due to such filterable viruses. Among them the most troublesome are in the veterinary field: foot-and-mouth disease, hog cholera, canine distemper, rabies, equine encephalomyelitis, equine infectious anemia, feline infectious enteritis, psittacosis, etc.; in man, infantile paralysis, small pox, influenza, measles, etc. Immunologists find their task increasingly difficult because the preparing of effective vaccines and antisera is hampered by the fact that viruses do not grow on the usual media employed for microbes.

■ Nevertheless very important achievements have been reached in the practical field. Among them only the new foot-and-mouth disease vaccine may be cited. This was prepared by adsorbing the virus on aluminum hydroxide and attenuating it by chemical methods which we have employed more and more since Ramon found the anatoxin (formaldehyde toxin).

The primary role of Goodpasture's and Woodruff's success in cultivation of certain viruses on the chorioallantoic membrane of the developing chick embryo has been stressed just recently. Frenkel showed that the virus of foot-and-mouth disease, which will not grow on this structure, multiplies on skin explantations of cattle, sheep, and hog embryos. These proceedings afford us means of fostering the growth of viruses with procedures closely related to cultural methods used for microbes. Along these same lines a vaccine against typhus has been prepared, the need of which more than ever is felt, because of the spreading of the disease among the armies in the present war and in the Polish concentration camps.

But are we not implying by such a comparison that viruses are in the domain of microbiology, a branch which concerns the life of small beings? Unwittingly we come to the vital question which has been haunting the minds not only of immunologists but also of biologists in general.

Undoubtedly if we concede that the spreading of infection is due to paracrystals like those Stanley prepared from tobacco mosaic diseased leaves, or to true crystals like that obtained by Bowden and Pirie

from an analogous tomato disease, we are deviating from the common concept of life. Let us examine then with serenity if the proofs furnished hitherto are sufficient for granting that the infective property resides in the innermost protein of those crystals or in the non-crystallizable proteins. The latter because of their physical and chemical behavior are placed on the same category as the crystallized proteins. Let us confess that the crystalline shape has not been of any use in the study of proteins.

■ The earlier belief that viruses consist of a lower group of microörganisms was given a severe blow by the discovery of Dr. W. M. Stanley of the Rockefeller Institute, Princeton Station. He proved that the virus which produces the mosaic disease of tobacco could be crystallized. What is more, when these crystals were put on tobacco leaves, the mosaic disease spread like a slow fire over the whole area just as if they had infected it. This shattered a laboriously gathered evidence of microbiology. The fact that these crystals behave in many respects like living beings still holds the attention of bacteriologists focused on this as yet unsolved problem.

But reflection is slowly appeasing the initial clamor upraised among the biologists. Although the fact rightly deserves our careful thought, no revolution has yet started to upset the solid ground on which microbiology and biology lie.

■ In the science of immunology and medicine we are aware of the great contributions chemistry has made in the preparation of antisera for use in the treatment of disease. Among these specialized methods is the precipitation of proteins by means of $(NH_4)_2SO_4$ for concentration of antibodies. Although these precipitated concentrates are considered by chemists to be as nearly pure as can be made with the finest techniques, the microbiologist who uses these preparations for injection into human beings, must consider them as raw, unpurified products. For use in medical treatment they must be biologically pure, with no contaminating microorganisms. Similar chemical methods have produced certain crystalline substances which simulate the filterable viruses, *e. g.* tobacco mosaic. Either the chemical agents themselves are the carriers of the disease, or virus impurities are present in these chemicals. An experiment of Flu seems to confirm this latter possibility, at least so far as bacteriophage virus is concerned. He isolated from a microbe (*Bact. megatherium*), by means of precipitation with $(NH_4)_2SO_4$, a globulin which, dissolved in saline solution, had no visible effect on the microbe. But this globulin when mixed with an active bacteriophage, even after 20 precipitations with $(NH_4)_2SO_4$, still had a lytic effect upon *B. megatherium*. These methods are similar to the ones used by Stanley for purification of virus crystals. Gratia and Manil found the same behavior in bacteriophage when added to the mosaic virus. After several recrystallizations the crystals still contained a considerable amount of bacteriophage. These experiments show the possibility of an absorption of impurities under conditions similar to those used by Stanley.

On the other hand, if actually the parent substances of viruses should be the normal constituents of the host cells, how could we explain the fact that virus diseases do not arise spontaneously from time to time in isolated communities? As Laidlaw pointed out, there is no evidence that they are ever created anew in this way. Bacterial cultures can be kept free from bacteriophage indefinitely without any trouble. Accidental con-

This is the last of a series of three articles by Prof. Ascoli in which he has endeavored to acquaint veterinarians with the brilliant research work in field of ultra-microscopic organisms, which has been carried out recently. In no other realm in biology is scientific knowledge advancing so rapidly as in the field of filterable viruses. The present period, in this field, corresponds to the last decade of the 19th century in the science of bacteriology, when discoveries far outran the employment of the new knowledge which research made generally and easily available.

tamination happens only when no precautions are taken. Great Britain is free from rabies as long as the quarantine laws are faithfully observed; outbreaks occur only when quarantine regulations are relaxed. America and Great Britain are free from foot-and-mouth disease, because by killing all the infected animals, the spreading of the virus is stopped at its first appearance. There is as yet no reliable evidence that viruses ever arise spontaneously in a normal host, and there is much to indicate that they always come from an outside source. As is well known, in Great Britain foot-and-mouth disease is immediately reported, and wherever an outbreak occurs the whole of the infected herd is slaughtered, the carcasses are burned and the stables disinfected. The movements of animals in the vicinity of an outbreak are watched carefully and a sharp lookout is kept to prevent the disease from spreading. Such a policy would not have succeeded in lowering the incidence of the disease if the virus arose spontaneously, as occurs in some malignant tumors which have an endogenous origin. Moreover, an autonomous outbreak of virus diseases has not had any experimental proof. On the contrary, their multiplication *in vitro* is receiving more and more experimental support.

■ Recent success in cultivating the small refractile granules which can be seen in virus supensions is again tipping the scales in favor of the view that viruses thrive on appropriate media in exactly the same way microbes do. And it is significant that these media are not broth, gelatin, agar-agar, potato or serum, but living tissue, and chiefly the chorio-allantoic membrane of the developing hen's egg. So it can be seen that for their growth these viruses need the presence of living cells. However, this need can be supplied by the simpler technique of incubating the viruses on minced embryo tissue in Tyrode solution. This procedure does not differ fundamentally from the requirements of certain microbes. Many microorganisms require in the culture media the presence of substances which they are unable to synthesize; for instance, the hemophilus group must have hematin; staphylococcus, nicotinic acid, and so forth. In the same way, it would seem as though viruses need some metabolic elements of a transient nature such as those which Neuberg was able to capture before they reached the final stage.

As mentioned earlier, Waldmann, Koebe and Pyl, by adsorption of the virus on aluminum hydroxide and attenuating it with formaldehyde, prepared a vaccine which seems to provide a good and lasting immunity against the disease. The fact that aluminum hydroxide adsorbs viruses helps to verify the assumption that they have a strong tendency to adhere to glutinous substances. Crystallinity in such cases offers no definite proof of the non-living nature of the material and is no absolute guarantee of purity. The discoverer of tobacco mosaic crystals himself now agrees with such a viewpoint. Even the retention of constant properties following repeated crystallization cannot put an end to debate. When members of the tobacco family, such as spinach or phlox, whose proteins show no serological relationship, are injected with tobacco mosaic virus, the same virus is produced. But this phenomenon supplies no evidence of its non-living nature. Diphtheria toxin, too, is built up by the diphtheria bacillus regardless of the kind of protein it has at its disposal in the broth in which it thrives. There is little reason, Stanley says in his latest article, to accept the results of the researches on protein viruses as demonstrating unequivocally that viruses are not living and are merely ordinary protein molecules. The physical and chemical properties are similar to those of ordinary proteins, yet the biological properties are quite different, because virus activity implies the ability to multiply, change, mutate and produce in some cases inclusion bodies in the infected cells as well as lasting immunity in recovered hosts. Therefore it is difficult to decide whether they are ordinary protein molecules or organisms, cells or some kind of particles on the borderline between living and non-living. Chemical analysis gives us no clearer concept of their structure. Tobacco mosaic and ring-spot viruses appear to be composed solely of nucleic and amino acids. In

the case of bacteriophage preparations only a small amount of phosphorus appears in the form of a carbohydrate containing nucleic acid; the linkage of the remaining phosphorus is unknown. After Koegel and Erxleben made the striking demonstration that glutamic and other amino acids found in cancerous tissues have markedly lower optical rotations, denoting the presence of unnatural isomers, it was logical to seek for such unnatural isomers among amino acids formed by hydrolysis of the nucleo-proteins believed to be tobacco mosaic virus. In Stanley's laboratory Doctor Ross determined the rotation of naturally-occurring d-glutamic acid. Hitherto chemical examination has failed to demonstrate any special compound resulting from the breakdown of virus proteins.

■ One would be tempted with Levaditi to differentiate viruses affecting animals or man from those affecting plants on the basis of the fact that crystallinity has been found only in the latter, while papilloma and all animal viruses could not be crystallized. Yet Stanley rightly points out that crystallization should be regarded as only one of many tests that may be used to determine purity by chemical means. All of these tests should be applied before a decision is reached. Neither a virus rendered inactive by formaldehyde nor the reactivation of a formalinized virus, both of which are accompanied by chemical changes, indicates more than a parallelism that can be considered equally well as a change in virus activity or a change in chemical properties. Since both processes are influenced in the same direction, one is not justified in considering such a correlation as a proof that the virus activity is a property of the nucleoproteins, nor can one discover anything concerning the structure necessary for their activity. There are prerequisites in biology as in chemistry which are essential and cannot be disregarded. Although biology does not give mathematical certainty, let us not overlook the postulates claimed in our case.

Stanley himself says that the time has come, or at least is rapidly approaching, when it can be said that virus activity is a specific property of nucleoproteins with the same assurance as when it is said that the properties of water are those of the water molecules, or that the hormone properties of insulin belong to the molecule known as insulin. The problem has thus been placed on a footing in which biology and chemistry have achieved their most marvelous results. Perhaps it would have been preferable to view this matter from a biochemical standpoint and to extend the comparison to vitamins. Are we in the researches on viruses, actually approaching the certainty we have when dealing with vitamins and hormones?

■ The immense progress, practical and theoretical, achieved by chemical synthesis in vitamins and hormones is in marked contrast to the progress made in virus studies, which are hampered by high molecular weight (17,000,000 for a molecule of a diameter of 35 millimicrons) and their comparatively large chemical structure. Analysis and synthesis of vitamins and hormones can be made because of their simple structure. The architecture of nucleoproteins is far from being understood. The method used in the studies of Fischer and Abderhalden on polypeptids did not bring the hoped-for results. It may be that through the studies of antibodies we may advance in this direction. The relationship between antibodies and serum globulins gives support to that hope. Experiments in this field may lead to an appreciation of the structure and mechanism of the viruses. Of course if the size and molecular weight of the viruses should be in the same range as that of the protein molecule and we cannot by any known chemical means separate the two components, the only alternative is to suppose they are one and the same thing. However, it is noteworthy that the only experimental evidence that has been brought to bear on the hypothetical identity of the two substances is through the dubious method of centrifugalization. It seems impossible with our present knowledge and methods to resolve the problem concerning the exact nature of the filterable viruses, whether they are living or non-living. Our appalling ignorance of what lies between life and death must "give us pause." It was Dante who said:

"Insane is he who hopes
Our reason may that infinite space explore."

Recent Materia Medica

IV. Vitamin E.

By PROF. VICTOR LEWITUS,
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History

LESS than 20 years ago (1921), Evans and Bishop discovered that rats subsisting on a diet containing adequate amounts of the then-known vitamins, grew normally and exhibited normal estrus cycles. Yet these animals were unable to carry through a normal gestation period because of the death and resorption of the fetuses. Upon the addition of extra amounts of vitamins A, B, and D, the condition was not at all corrected; and hence the existence of a new fat-soluble vitamin was suspected. Not long afterward, Evans and Bishop proved the presence of this vital factor in wheat germs, lettuce leaves, and other sources. The work of Mattill, Sure and others confirmed this early finding and it was not long before biochemists began to search for the active fraction of wheat germ oil. In 1936 Evans and Emerson succeeded in isolating three crystalline derivatives from the non-saponifiable portion of wheat germ oil. The active portion of one of these, called alpha-tocopherol, proved to be so potent that even a single injection of three milligrams carried rats on vitamin-E deficient diets through a normal gestation period. Of the other compounds, one was only slightly effective and the other completely inactive. Evans and his co-workers were also able to isolate the active compound from other sources. In 1938, Karrer and others announced the successful synthesis of alpha-tocopherol. It has been shown that the synthetic product is as potent biologically as natural alpha-tocopherol, and now that the pure substance has become available in the form of a more stable substance — alpha-tocopherol acetate—one may anticipate clarification of many unsolved problems regarding the physiological functions of this most interesting vitamin E.

Sources

Vitamin E is widely distributed in natural foodstuffs. Most animal tissue contains limited amounts, but green leafy vegetables such as lettuce, spinach and water cress contain liberal quantities. Perhaps the richest natural sources are seeds and cereals, the vitamin being present almost exclusively in the embryos. For this reason vegetable oils such as corn oil, cottonseed oil and palm oil yield large amounts, and the most potent natural source is wheat germ oil. Egg yolk and milk are also good sources of the vitamin. The non-saponifiable fraction of wheat germ oil is one of the sources of artificial concentrates. but the most recent artificial material is alpha-tocopherol acetate, which is now being produced on a commercial scale.

Chemistry and Properties

The vitamin E complex may be considered to consist of three components present in wheat germ oil, namely, alpha-, beta-, and gamma-tocopherol. Of the three, alpha-tocopherol is most active. The tocopherols are alcohol-like in chemical structure, and are fat-soluble. They are resistant to heat, but sensitive to ultraviolet light, and are readily decomposed in the presence of rancid fats. For this reason it has been found that substandard or stale vegetable oils often lose their vitamin E potency; and when vitamin-E free diets are wanted for experimental purposes, fats that readily rancidify are mixed with foods to insure the absence of the vitamin. Since vitamin E is so susceptible to oxidative destruction, and since it is capable of being esterified easily, a more stable combination has been sought. The acetyl ester, alpha-tocopherol acetate, reported by Isler late in 1938, appears to have as pronounced an activity as alpha-tocopherol itself, and possesses the advantage of greater stability. Alpha-tocopherol has the formula $C_{29}H_{50}O_2$. It is a yellow, viscous oil, freely soluble in organic solvents but insoluble in water. The acetate derivative is both dextro- and levorotatory. Since the work on wheat germ oil

has furnished the three tocopherols, it has been determined that cottonseed oil and palm oil have only alpha- and gamma-tocopherols. Lettuce is found to contain only alpha-tocopherol. It has been shown that each of the tocopherol compounds possesses antioxidant properties, but their degree of protection is not proportional to their biological activity.

Assay or Standardization

No official standard to designate vitamin E potency has been adopted, as yet; but it is quite likely that the effects of pure alpha-tocopherol will be taken as the measure of activity. Thus far, the standards have been based on the activity of the amount of certain refined wheat germ oil "necessary to reëstablish normal gestation in female rats in which resorption of fetuses has been established as a result of deprivation of vitamin E, under the conditions of the bioassay described by Evans and Burr." Thus, one "Evans unit" is the amount of vitamin E just sufficient during the gestation period of previously non-productive female rats to produce a litter. Another unit is the Pacini-Linn unit expressed by the quotient from the division of 1,000 by the number of milligrams of material under test required daily during the 21-day gestation period of the rat to produce a litter. As has been stated, neither of these has been accepted officially. The approximate equivalences of wheat germ oil and the tocopherols are: 500mg crude wheat germ oil equals 25mg non-saponifiable fraction, equals 5mg beta-tocopherol, equals 3mg alpha-tocopherol.

Functions of the Vitamin

Vitamin-E deficiency produces characteristic types of sterility in male and female rats, mice, chickens and other animals. In the female rat the estrus cycle, ovulation, and fertilization proceed normally in the absence of the vitamin; in fact, no detectable abnormality has been observed in the maternal organism. Pathological development in the embryo and the embryonic contributions to the placenta, which prevent successful implantation, are observable about the 8th day in the case of rats. It has been found that normal implantation may occur if sufficient doses of vitamin E are given even as late as a few hours previous to implantation. Urner has reported normal development of the embryo up to the tenth day. At this period of E-avitaminosis, hemorrhage into the amniotic cavity, rarefaction of the mesenchyme, and failure of development of the vascular system produce death of the fetuses, which are soon reabsorbed. Since the maternal decidua appears to be normal throughout, it is indicated that the failure of implantation and subsequent death of the fetus is not due to abnormalities within the uterus or the mother herself. A parallelism in the case of chickens may be drawn. When chickens receive foodstuff inadequate as regards vitamin-E content, they continue to lay eggs which, however, are incapable of hatching. Adamstone has shown that embryonic death usually occurs about the fourth day. This is attributed to the hemorrhage and failure of vascularization due to the development of a "lethal ring" of proliferative tissue within the blastoderm. A close correlation between the vitamin-E content of the hen's ration, the vitamin-E content of the eggs, and the hatchability of the latter has been demonstrated by Barnum. This emphasizes the necessity of vitamin E for reproduction in chickens.

In the male, the changes due to vitamin-E deficiency are much more profound than in the female. Although the reproductive system of females is unaltered, testicular degeneration is one of the prominent effects in males when this vitamin is lacking. The resulting sterility is irreversible, in contrast to that in the female. Immotility and clumping of the sperms is first observed, followed somewhat later by degeneration of the germinal cells (both seminiferous epithelial cells and spermatozoa) of the testes—an effect which is found in rodents as well as in chickens. In other words, in the female the placental function fails, whereas in the male, degenerative changes take place in the testes. Since such extensive changes resulting in functional castration occur in the testes, it is reasonable to expect effects on the pituitary glands. Nelson and Stein, and Van

Wagenen have reported just such changes similar to those following actual castration; and as might be anticpiated, no such pituitary change is demonstrable in females since no degeneration of ovarian tissue takes place; however, it has been reported by Rowlands and Singer that the luteinizing hormone is present in subnormal amounts in the latter animals. Other endocrine aspects of the problem have been studied by many workers, but conflicting evidence has thus far been presented.

Data derived from other experiments, including the original work of Evans and Burr, indicate that a deficiency of vitamin E is manifested also as a retardation of growth in rats. Since this defect appears only later in the rat's life, it has been overlooked by many workers in their experimental studies. Olcott and Mattill, Martin, Blumberg and others have shown that late retardation of growth in rats deficient in vitamin E, can be cured or prevented by administering wheat germ oil. Since wheat germ oil contains other biologically active components than vitamin E, the later work of Evans and Emerson with highly purified alpha-tocopherol is significant. They showed that when this supplement is added to the diet, it causes resumption of the growth in vitamin-E deficient rats.

Besides the failure of reproduction and retardation of growth, other symptoms have been noted by various authorities in the field. Evans and Burr, for example, in 1928 reported a characteristic paralysis in rats due to sub-optimal amounts of vitamin E. This condition was found to be curable if the mothers were fed adequate vitamin E during pregnancy, but not if the material was fed to the paralyzed offspring. The condition, studied by Olcott, appears to be due to degenerative changes in the striated muscles, with no detectable neural lesions. When the condition occurs in adult rats, the syndrome is also incurable. The condition of muscular dystrophy has also been reported in rats by Evans and others; and more recently Goettsch and Pappenheimer have shown that muscular dystrophy may be induced in rabbits, guinea pigs and ducklings by restricting the vitamin-E intake. Only partly successful results have been obtained with the administration of wheat germ oil to cure this condition; some workers obtained favorable responses with the whole wheat germ itself. This indicates the necessity of more than one factor for these complicated conditions, again stressing the possible interdependence of the several essential vitamins in the animal economy. Evidently, a combination of vitamin E (or the vitamin-E complex) and vitamin B (or the vitamin-B complex) are necessary, since both fat-soluble and water-soluble components of the wheat germ appear to be curative in their effects. Pappenheimer and Goettsch have shown another condition which may be caused by lack of vitamin E, namely nutritional encephalomalacia in growing chicks. These animals show tremors, ataxia, twisted neck, clonic spasms, and stupor, and the condition often ends in death. The lesions found at autopsy occur most often in the cerebellum, but may also be found in the cerebrum, brain-stem and medulla. These consist of edema, degenerated cells and fibers, small hemorrhages, and capillary thrombi. The degeneration is due to ischemia. At first believed to be caused by lack of certain of the B vitamins (*e.g.* B_4), it has more recently been shown by Dam, Glavind and others that vitamin E in the form of synthetic alpha-tocopherol prevents this very grave condition in chicks.

Although a close connection exists between nutritional encephalomalacia and naturally-occurring "crazy chick disease," both due to vitamin E deficiency, similar deficient diets produce totally different effects in other fowl. In the duckling the condition is characterized by an extreme, progressive myasthenia which ends fatally within a few days. The skeletal muscles show widespread hyaline necrosis of the fibers, the central nervous system being unaffected, unlike that of the chick. In the turkey, still another condition is recognized on E-deficient diets. A selective necrosis of the gizzard muscle develops, the skeletal muscles and the central nervous system remaining intact. This emphasizes the variation in response to vitamin E deficiency of the several species.

Experimental studies by Einarson, Ringsted, Bicknell and others in human diseases indicate that vitamin E therapy is likely to be of value in all conditions involving degeneration of the muscular or nervous systems. The quantity of vitamin E in the average human diet is such that many persons may be on the edge of a vitamin-E deficiency; this may become serious if for any reason absorption from the intestine is impaired, or if the demands of the muscular and nervous systems are higher than normal from a hereditary disposition or from the toxic influence of certain diseases. It is possible, for example, that tabes dorsalis is the result not of syphilis alone, but also of a deficiency of vitamin E, causing degeneration of nerve tracts already weakened by syphilis. Working on this theory, muscle dystrophies in children and amyotrophic lateral sclerosis in adults were treated by Bicknell with whole wheat germ, with encouraging results. Recent work by Wechsler with wheat germ oil as well as ephynal (alpha-tocopherol acetate), also points to vitamin E as a possible cure of amyotrophic lateral sclerosis.

Sterility resulting from vitamin-E deficiency has been definitely observed in rats, mice and chickens. The species response to the vitamin E is extremely variable. Numerous reports have appeared which indicate that wheat germ oil may serve as a prophylactic in possible cases of abortion in farm animals; but the question of the necessity for using vitamin E for normal fertility in these animals has not altogether been settled. The same statement holds for human beings, vitamin E as yet being considered in the experimental stage in the therapeutics of abortion or of certain types of human sterility.

Since vitamin E is readily stored in the body, the levels at which it is stored are an important consideration. Furthermore, Goettsch has very recently stated that "some animals may be able to synthesize vitamin E from precursors." Hence, it is small wonder that such enormous species variations are possible, similar to those of vitamin C. This may account for the different results obtained with the different animals not only with vitamin E but with practically all the members of the vitamin family.

Practical Applications

The use of refined wheat germ oil or wheat germ oil concentrates has been attended by encouraging results. However, some of the work thus far done must be considered in the experimental stage, as has already been pointed out, for there are several factors other than vitamin E in the natural products. The value of all the work thus far reported will be confirmed, perhaps, when the recently isolated or synthesized products have been tested over long periods of time and upon numerous species of animals.

Card has conclusively shown that if vitamin E is greatly lessened in the diet of laying hens, eggs produced from such hens fail to become fertilized or to hatch, but that if hens are given adequate amounts of the vitamin, the eggs subsequently produced are fertilizable and hatchable. The response seems to be very prompt when vitamin E is supplied. Adamstone has shown that chicks die on the 4th day of incubation, just before the reproductive system begins to form, if they come from hens fed E-deficient diets. He also showed that chicks escaping the 4th-day death may succumb at the 15th to 18th day stage of development, also probably because of vitamin E deficiency. Should the chick survive, it will be born stunted, weak, and unsalable. It is therefore clear that for normal hatchability, vitamin E is one of the essential supplements that are required in the feed of laying hens. Some types of range paralysis appear to be associated with vitamin E deficiency as shown by cures obtained with vitamin E in Butler, Warren and Pacini's work with fowl paralysis. Mackenzie and McCollum have recently shown that vitamin E will correct muscular dystrophies in rabbits. Reports on the treatment of lymphomatosis in chickens with vitamin E or wheat germ oil have been contradictory.

Dam and Glavind have just reported experiments on chicks. Extracting completely the protein contained in their diets, they in-

duced an alimentary exudative diathesis which could be prevented by feeding alfalfa and certain alfalfa extracts. Wheat germ and hips that were dried, constituting 5% of the diet, were effective as anti-exudative factors. Vitamin E, in the form of alpha-tocopherol or the acetate, was also found to have definite anti-exudative properties. This appears to be a novel method of securing an assay of vitamin E potency. Alimentary exudative diatheses are brought about, according to the authors, as a result of inflammation and hyperemia of the capillaries of the connective tissue, particularly those of the adipose tissue. With this explanation it becomes a bit clearer just why vitamin E or wheat germ oil should be so effective.

A number of authorities have obtained favorable results with vitamin E supplements in the case of dairy cattle. Vogt-Müller and Tutt have reported a definite response in sterile cows to treatment with wheat germ oil. For example, Vogt-Müller obtained 49 living births from 70 previously sterile cows. He likewise obtained litters from 70% of previously sterile sows after giving wheat germ oil. In some instances, the breeders have injected the animal with the oil intramuscularly. A saturation method of dosing cattle has been suggested by Pacini in which he claims success in 85% of the cases (in place of 70%) using wheat germ oil in massive doses. Moussu, in France, has reported success in the prevention of epizootic abortion in cows, so prevalent in European countries. In experimenting upon about 7000 cows he demonstrated that three subcutaneous injections of 30 to 40cc of wheat germ oil suppress abortions of this type in 98% of the cases. Moussu believes it is a disease of the fetus; that deficiency in vitamin E results in lowered resistance of the fetal tissues, and thus permits secondary infection by the microorganism. Speaking of the method of treatment he states: "The method is without action on *Br. abortus* but has its action exclusively on the fetus and fetal membranes which are influenced to resist the presence of *Br. abortus* or other organisms that are harbored where abortion disease is epizootic." It should be noted that many authorities do not favor injection of the oil. Hofstra has pointed out that common forage plants usually contain sufficient amounts of vitamin E. Cows fed industrial by-products or forage which has come from excessively fertilized soil often suffer vitamin E deficiency. Quin has pointed out that maximum results obtained from wheat germ oil can be obtained only when it is backed by rations yielding adequate amounts of vitamins A and B. "During the drouth of 1934," he states, "farmers of southern Iowa noted a marked failure of proved cows and heifers to conceive when bred the next spring. After grazing on the normal summer pastures in 1935, these same animals conceived readily without supplemental treatment—a rather clear-cut example of functional sterility due to vitamin deficiency." The recent work of Verzar has pointed out that enormous doses of vitamin E are required to cure creatinuria in animals, whereas only small amounts secure prophylaxis. He attempts to point out the hormonal relationship of the vitamin E effects. More recently, Drummond, Noble and Wright have attempted to disprove any relationship between vitamin E and the endocrine system directly. They conclude that "Vitamin E when administered to immature female rats or to hypophysectomized adult female rats did not exert any effect on the ovaries, uterus or vagina. The pituitary glands of E-deficient male rats were found to contain an increased amount of gonadotrophic hormone. Glands from E-deficient female rats showed a decreased amount of luteinizing hormone when tested on immature hypophysectomized rats . . . The suggestion that the effects of vitamin E deficiency are produced by hormonal imbalance are not supported by the experimental evidence described." Mason has just recently stated that vitamin E may be concerned in the synthesis of certain components of the chromatin molecule. He considers the function to be related to the nuclear activity of cells, especially those undergoing unusually rapid proliferation and differentiation (germ cells of testis, developing embryo). He discounts the attempt to relate the vitamin E functions to the endocrines directly.

Two Collection Ideas That Click

By FRED E. KUNKEL, Washington, D. C.

BELIEVING that return envelopes get bills paid quicker, one veterinarian adopted the plan of enclosing a reply envelope with each bill, printing the envelope with his name and address on the mailing side, and with four words on the flap reading, "Our Story Is Inside."

When the flap is opened, this printed message is found underneath:

OUR INSIDE STORY

One of our ideas about being in business is to be as helpful as we can to our customers. We even help them pay their bills by sending them handy return envelopes like this one.

Won't you slip your check into this envelope to balance your account and help us with our collections? Thank you!

Underneath that, on the envelope (over which the flap fits, sealing it out of sight when mailed) is this:

MEMO STATEMENT
Of Your Account
Balance Due.....$.....
THANK YOU!

Since putting this plan into effect, the veterinarian's collections have increased noticeably and returns stream in much quicker than ever before. Not everybody keeps a supply of envelopes around the house, and by furnishing them himself he removes one reason for failing to send in the remittance.

Carrying this idea a step farther, a "business reply" envelope, which requires no stamp, would appeal to those who do not have a stamp handy—or to those who have stamps, but whose instincts of economy are offended at the idea of wasting two or three cents just to pay a bill.

Another veterinarian, who was at his wit's ends as to how to collect some long outstanding accounts, without offending the clients or writing them strong collection letters (where no attention had been paid to ordinary letters), realized one day that debtors, in general, don't write in telling why they can't or don't pay, unless they have the money to send along with that letter.

So, instead of sending out another letter, this veterinarian hit upon the idea of sending them a "Call Card," on which was printed in large, heavy type, "Please phone 2345," without any name on it or any other message or return address.

In nine cases out of ten, these people actually call up just to see who sent them that card. Then, when he has them on the telephone, he can straighten out the whole thing. This also takes the sting out of his calling them up first to ask for payment of his account—an act which would undoubtedly antagonize them.

By using this idea regularly now his unpaid accounts no longer reach the stage where strong letters have to be written. Strangely enough, the card is just as effective the second or third time (if they get behind again) as in the first place.

They do not call up this time, but in most cases they send in their payment right away.

"This method is a cheap way of sending collection mail," he says, "and it is pretty effective, considering results. This "Call Card" opens the way to a direct communication so we can talk to each other.

"I recommend not sending out more than 25 postals a day, however, for the first time I tried it I sent them out to every delinquent account on a Thursday, and on Friday and Saturday the telephone was ringing all day long. More than two-thirds of them called up, including a lot of accounts to which I was about to send the strongest kind of a collection letter, and a few I had about decided to turn over to a collection agency. They called up and explained their circumstances, or told me when they would come in with the money, and they kept their promises in 95% of the cases."

Shigella Equirulus a Factor in Foal Ill

February 28, 1940, a black, 14-year-old western-type mare, heavy in foal, was presented for observation at the veterinary clinic of Kansas State College.

The mare had been bred to a spotted western stallion, weighing approximately 1,000 pounds, on March 6, 1939. This made the period of gestation, including the day the mare was admitted, 360 days, or approximately 14 days over the 346 days which is usually considered the upper limit for the equine gestation period.

Because of extreme nervousness of the mare the temperature and pulse were not obtained, the clinician deeming it advisable not to excite her any more than necessary.

The mare appeared normal on cursory examination, although the abdomen was greatly distended. Milk was present in the udder. The animal was confined in a roomy, well-bedded box stall to await parturition.

The mare foaled at 9:00 p.m., February 29th. The birth was unassisted. The fetal membranes were released readily and normally. The non-gravid horn of the placenta was observed to be somewhat heavier than normal, but in other respects the membranes were normal.

The foal was weak from the start, although it was able to stand and move about the stall. The first morning it could not suck and had to be helped. Treatment was then instituted, consisting of the intravenous administration of 500cc of whole blood and 150cc of dextrose.

There was a slight impaction of meconium, which was removed manually. To relieve constipation, two ounces of olive oil were given in the second feeding, which consisted of mare's milk and lime water.

The second day the foal had to be held to suck and was noticeably weaker. By 10:00 a. m. it could not support itself and had to be assisted. The temperature, taken shortly after noon, was 102°F.

From noon on the foal became rapidly weaker, being completely prostrated and unable to rise, and died at 6:00 p. m., or 45 hours after birth.

On post-mortem examination mild peritonitis and pleuritis, with a slight amount of ascitic exudate in the thoracic cavity and a considerable amount in the abdominal cavity, were found. A few petechiæ and ecchymoses were present on the pleura of the lungs and on the heart. A marked enteritis was present throughout the intestinal tract, while the stomach was almost normal with only a very slight gastritis. In the region of the kidneys there was edema and infiltration of exudate in the tissues. The capsule was firmly adhered to the kidney and peeled with difficulty. Examination of the surface of the kidney disclosed numerous small abscesses scattered irregularly. Incision of the kidney showed that the abscesses were restricted mainly to the cortex. The liver presented a few small abscesses beneath the capsule. The umbilical vessels and urachus were apparently normal in all respects. The joints were slightly swollen.

The kidneys and liver were taken to the pathology laboratory, where cultures were made of the abscesses. *Shigella equirulus* was isolated.

It is interesting to note that this organism has been the subject of considerable research in Kentucky, where it is quite prevalent. It has been recognized under several other names, including *Bacillus nephritidis equi, Shigella viscosum, Bacillus equirulus, Bacterium viscosum equi.* When this organism is present the majority of foals die on or before the third day following parturition, the average age at death being 2.9 days.

Practically all foals that die are visibly affected at birth and are often unable to stand. The illness is characterized by a sudden onset and complete prostration, with death following rapidly. The pulse, respiration and temperature are usually increased during this condition. Often the joints are noticeably enlarged. An almost constant post-mortem lesion is the abscessation in the cortical layer of the kidneys.

It is possible that this organism is present in other cases of foal ill in the middlewest, but is not discovered because of the lack of facilities for culturing and identifying it in the field.

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Ventro-Lateral Hernia in a Zebra

December 23, 1939, a Chapman's zebra (*Equus chapmani-burchelli*) was presented for treatment at the veterinary clinic of Kansas State College by the owners of a small circus whose winter quarters were in the nearby town of Emporia, Kans. The value of this animal, the first of its kind in the history of the clinic, was estimated at $1,000 by the owners, who advertised it as "the only trained zebra acting with an elephant in the United States."

History.—The animal, a female zebra about 15 years of age, had fallen on a stake about five months previously. This accident resulted in the appearance of a small but gradually enlarging swelling in the region of the right flank. The animal was not impeded by the condition, and continued to appear in the circus act. As the swelling increased in size, however, treatment was deemed necessary, and the animal was brought to the clinic.

The zebra was tractable and easily managed in the presence of her companion, a dwarf bay mule, which was therefore allowed to accompany her into the clinic. She was cast on the large-animal table and securely restrained for an examination conducted by Drs. Frank and Roberts. A firm, fluctuating swelling was palpated externally; internal palpation revealed a longitudinal opening, about one inch by four inches, just anterior to the fold of the right flank. A diagnosis of ventrolateral hernia of the small bowel was made, and a guarded prognosis given.

Treatment.—The skin over the swelling was shaved and the patient was tied in a well-bedded box stall without food during the night. Cotton packs saturated with an alcoholic solution of bichloride of mercury were held in position by a wide muslin bandage over the operative area during the night.

The following morning the zebra was restrained on the large-animal table and anesthetized with 375cc of a 6% solution of chloral hydrate, administered intravenously. This produced complete narcosis. The operative area was cleaned with alcoholic bichloride of mercury solution (1:1000), and a sterile operative cloth was placed over the area. A vertical incision was made over the swelling and the opening was

The patient when presented for treatment

spread by means of traction upon four linen sutures placed about one-quarter inch from the edges of the incision. Adhesions which had formed between the skin and the hernial sac were broken down by blunt dissection with the fingers. Some of the strong adhesions were cut; the slight hemorrhage was controlled by hemostats. During this procedure 125cc of 6% chloral hydrate was administered intrajugularly. A purse string suture, using No. 20 catgut, was introduced loosely, and the hernia was gradually reduced by manual pressure on the outside and traction from within per rectum. The suture was then tightened, closing the hernial ring. The cavity was flushed with tincture of zephiran and tamponed with cotton. Continuous linen sutures were used to approximate the edges of the skin incision, and a ventral oblique incision was made through the skin at the postero-lateral aspect of the sac to permit drainage. Five hundred cubic centimeters of a 25% dextrose solution and one liter of sterile normal saline solution were administered intravenously. Flexible collodion was applied to the line of the incision except at the lowermost point where drainage occurred. The patient was allowed to remain

on the table until consciousness returned, then tied, watered, and bedded down for the night.

Twenty-four hours later the patient was observed to have a slight rise in temperature and to exhibit symptoms of a circulatory disturbance, with cold extremities. A capsule containing 250 grains of sulfanilamide was given per os. In five hours the temperature had dropped one degree, although the extremities remained cold to the touch. Feed and water were given and the animal was left alone. Several hours later it died.

Post-Mortem Findings.—No significant lesions were found except a slight area of congestion surrounding the incision just beneath the skin. This was to be expected because of the extensive fibrosis encountered at the time of the operation. The incision was healing properly, and there was no evidence of early peritonitis within the abdominal cavity.

Remarks.—The negative findings on autopsy and the symptoms of circulatory disturbance following the operation point to the probability that death was due to so-called secondary shock, the immediate cause of death being respiratory failure following changes in the central nervous system.

Although little is definitely known regarding the pathology of shock, the "traumatic toxemic" theory is perhaps most widely accepted. This theory postulates that some toxic substance alters the normal tone of the highly vascular capillary bed so that the visceral capillaries are filled at the expense of the peripheral and cerebral circulation; this would account for the drastic alterations evidenced by acute metabolic disturbance in several systems of the body. This theory gains credence through the fact that similar effects have been clearly produced by the injections of histamine into the body. Although there is no proof that this condition can occur in Equidae, the absence of lesions in this interesting case makes it appear likely that death was brought about in this fashion.

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Cesarean Section in a Cow

Cesarean sections performed at the termination of gestation are now a common occurrence. A little on the unusual side is an incident in the practice of Dr. C. H. Case, of the Akron Veterinary Hospital in July, 1939. At this time a cesarean section was performed on a three-year-old Guernsey heifer nine months after the normal calving date.

The patient was born July 16, 1936, and bred for the first time January 5, 1938. On March 22, 1938, Doctor Case in his monthly herd inspection found the patient pregnant. In August and September the cow began to jump fences and stray several miles. Parturition should have occurred early in October. Doctor Case examined the cow on October 22, at his monthly herd inspection, and questioned whether the fetus were alive. In November he again examined the cow, but refrained from attempting an abortion because the fetus was quite large. An attempt was made to express the corpus luteum in December, but this failed. During the following six months Doctor Case tried unsuccessfully to express the corpus luteum at each monthly herd examination. In June, 1939, hopes of aborting the cow were abandoned and a cesarean section was performed by Dr. L. K. Firth of the Akron Veterinary Hospital.

The patient was admitted to the hospital June 30th and fasted until the day following the operation, July 3rd. On Sunday morning (July 2nd) the assistants clipped an area posterior to the rib, and ventral to the lumbar processes, taking in the entire flank. The area ventral to the lumbar processes, posterior to the left side of the costal arch, and anterior to a vertical line through the tuber coxae, was shaved.

The cow was given a general anesthetic of chloral hydrate (two ounces in 12 ounces of distilled water) intravenously and placed in right lateral recumbency. The operative area was thoroughly cleansed with ether and disinfected with tincture of iodine. The cow was then covered with sterile muslin.

A 14-inch incision was made six inches ventral to the lateral processes of the lum-

bar vertebrae and midway between the tuber coxae and the last rib. The line of incision was directed downward and slightly backward. The incision was continued through the skin and muscles to the perito-

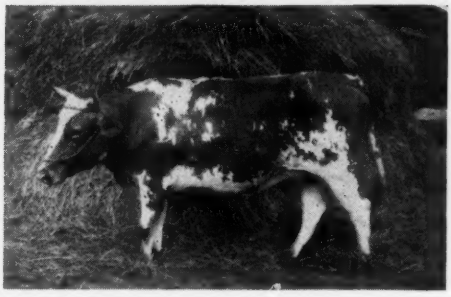
The mummified fetus was black and hard

neum, which was picked up with forceps and opened with scissors in a line corresponding to the incision. (This type of incision leaves more room and eliminates pockets.) Sterile gauze, eight inches wide, was clamped to the peritoneum and laid over the edges of the wound for protection against trauma and infection.

The uterus was grasped and gently lifted from the abdominal cavity. It appeared to be normal in color. The fetus occupied the left horn. A 10-inch incision was made through the dorsal wall of the uterus—the dorsum being used to lessen the likelihood of leakage and adhesions. Being careful not to contaminate the operator's hands or the incision, an assistant pulled the mummified fetus through this incision and swabbed the lumen of the uterus with sterile gauze. The uterus was then covered with moist sterile gauze, leaving only the incision exposed. A double row of Lembert sutures of No. 5 black twisted silk was used to close the incision in the uterus, which was then replaced in its normal position in the abdominal cavity.

The gauze packs around the edges of the wound were removed and the peritoneum grasped with artery forceps at three-inch intervals. A No. 9 black twisted silk suture was used to close the peritoneum, using a continuous suture with a lock suture every two inches. Interrupted sutures at one and one-fourth inch intervals of the same type of material were used to close the aponeurosis of the internal oblique muscle. The aponeurosis of the external oblique muscle was closed with a continuous suture, with a lock suture every two inches. The skin incision was closed with a mattress suture, using umbilical tape.

By the time the last suture was tied the patient began showing evidence of coming out of the anesthesia. In a short time she



The patient recovered without complications following the operation

rolled up on her sternum, and within an hour got to her feet.

The mummy was intensely black and weighed six pounds. It was quite dense, requiring some pressure by the thumb nail to make an impression on its surface. Oval spots on its smooth surface indicated where the cotyledons had been attached.

The patient was sent home in four days and recovered without complications. Doctor Case removed the mattress sutures on July 22nd. The other sutures were not removed.

The patient was bred after a convelescence of nearly three months. She came in heat again 22 days later and and was bred again. In December, two months later, Doctor Case diagnosed her as pregnant, confirming his diagnosis in each of the following two months. She is due to calve in the latter part of July and from all indications should have a normal parturition.

FRANCIS M. KENNEDY.
Lawrence, Kans.

Chronic Endocarditis in a Cow

March 7, 1940, a three-year-old Holstein cow was presented at the veterinary clinic of Kansas State College for treatment.

History.—The cow had calved one month previously. A poor appetite, loss in flesh, and decrease in milk flow had been noticed since freshening.

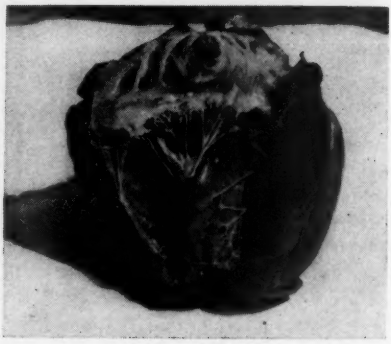
Symptoms.—The temperature was 103°F.; pulse, 105 beats per minute; and respirations, 19 per minute. The mucous membranes and conjunctiva were anemic. There was atony and impaction of the rumen. The blood picture was as follows:

Total white blood cell count	11,000
Hemoglobin (grams per 100cc)	7.5
Differential white cell count:	
Eosinophiles	1
Basophiles	0
Myelocytes	0
Juveniles	1
Stab	19
Segmenters	42
Lymphocytes	
Large	11
Atypical	13
Small	12
Monocytes	1
Total	100

Diagnosis.—On the basis of the fast pulse and the blood count a tentative diagnosis of traumatic gastritis was made.

Treatment.—It was decided to perform a rumenotomy. The area in the paralumbar fossa was shaved and disinfected. A local anesthetic of 1% procaine hydrochloride was used to anesthetize the skin, muscle, and peritoneum. An incision about seven inches long was made through the skin and subcutaneous tissue, starting about two inches below the transverse process of the lumbar vertebrae. The incision through the external and internal oblique muscles was made parallel with the fibers and long enough to provide space to work in. The incision through the peritoneum was made and four sutures were placed in the wall of the rumen, two on each side of the proposed incision. By means of these sutures, a portion of the rumen wall was pulled to the outside and a six-inch incision was made. An embroidery hoop fixed over a hole in the center of a sterile rubberized cloth was placed in the rumen and the protruding cloth so arranged as to prevent contamination of the incision.

About one-half of the rather hard and dry contents of the rumen was removed.



There were fibrinous-like ulcerative growths on the auriculoventricular valves

Two staples and three pieces of baling wire about two inches long, one partly embedded in the muscular wall, were removed from the recticulum.

The incision in the rumen wall was closed with a double row of Connell sutures, using No. 4 chromic catgut. The peritoneum was closed with continuous catgut; the muscle layers with continuous catgut; and interrupted mattress sutures of umbilical tape were used to close the skin.

The cow was discharged from the hospital and for the following two weeks showed an improved appetite and increased milk flow, but the pulse rate continued fast. The third week following the operation the cow had a relapse and went off feed, the milk flow diminishing again. A muffled sound was heard over the heart and respirations were increased. Diarrhea was present also. On March 29th the animal went down and was unable to rise. She died on April 7th.

Post-mortem lesions included cloudy swelling of the liver, hypostatic congestion of the lungs, fluid in the pleural and peritoneal cavities, chronic circumscribed peritonitis, and adhesions between the reticulum and diaphragm. The uterus appeared to be normal. The lesions in the heart were fibrinous-like ulcerative growths on the

right and left auriculoventricular valves, being most pronounced in the right valves. The growths were one-half to one and one-half inches thick.

C. K. WHITEHAIR.

Abilene, Kans.

Severe Burns in a Bull

History.—June 7, 1939, at Warrensburg, Illinois, a call was received saying that a bull had been burned. Upon arriving at the farmer's place, we found that an eight-year-old Jersey bull had been chained in a burning barn. Because of the intense heat, the farmer had been unable to free the animal; it had finally broken loose itself, but not before receiving severe burns.

After the animal had quieted enough to be handled, an examination showed large areas of hair scorched from the head and body. The hair that was not burned could be pulled out as from cooked flesh. The largest area not burned was the abdomen and legs. The following morning the bull's body was practically one solid blister, serum having collected beneath the skin. In a few weeks the burned areas had peeled from the head, back and sides, leaving the flesh beneath raw and exposed.

Treatment.—A 2% solution of tannic acid was first applied to the burned areas with cotton. An ointment of 2% yellow oxide of mercury was used in the eyes to sooth and protect the eye membrane and lids. The second day the following expectorant was administered:

℞

F. E. Belladonna	(one oz.)
Anti. et potassii tart	(four drams)
Syrupi	(one pint)

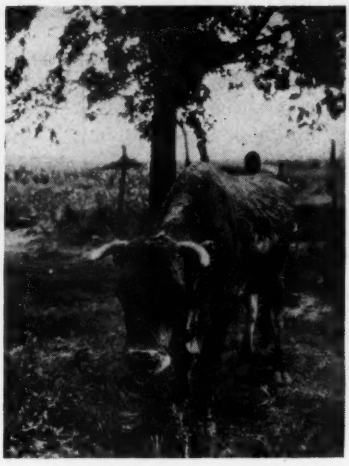
M.

Sig. One ounce every two hours.

This was indicated after the exposure of the lungs to the heat and smoke of the fire. To stimulate healing, scarlet oil was alternated with pyoktanin solution and a commercial healing powder. The raw areas were kept covered with vaseline. Screw worms in some of the raw areas were killed with chloroform and removed.

Intravenous injections of physiological saline solution were not used in this case, although they might have been of value.

Course.—By the first of September the bull was making a slow recovery. The sides were healed but the back was still raw. The animal was active, eating well and even



The bull was badly burned

showing a desire for service. Since recovery was so slow, and since most of the herd were daughters of the bull, so that its use for breeding would be limited in any case, the farmer decided to destroy the animal, although complete recovery appeared probable.

BUFORD D. PHILPY.

Manhattan, Kans.

In addition to being a source of annoyance to domestic animals, the stable fly, when present in large numbers, has a deleterious effect upon them owing to the quantity of blood drawn, and animals not infrequently are so reduced in vitality as to permit certain diseases to become acute and cause their death. In Africa and other parts of the world this fly is capable of transmitting certain serious maladies of horses and camels. In our country it probably plays a part in the dissemination of anthrax, and investigations indicate that the disease of horses known as swamp fever or infectious anemia is carried by it. Work thus far conducted indicates that the fly acts solely as a mechanical carrier, passing these diseases directly from one animal to another in biting.—*U. S. D. A. Bull.* 1097.

Phenothiazine for the Removal of Heterakis Gallinae from Chickens[1]

By ERNEST C. McCULLOCH and LYLE G. NICHOLSON, Pullman, Washington
Division of Veterinary Science, State College of Washington

NO anthelmintic has been previously reported that would safely and effectively remove the cecal worm, *Heterakis gallinae*, from poultry. Gildow, Lampman, Moore, and Holm[2] stated: "There is no satisfactory general treatment for cecal worms." Graham, Torrey, Mizelle and Michael[3] in their bulletin concluded: "No treatment for cecal worms has proved entirely satisfactory, for the location of these worms makes their complete removal practically impossible." The wide distribution of this parasite, the fact that it causes typhlitis when present in large numbers,[2] and its role as a vector of *Histomonas meleagridis*,[4] the etiological factor of enterohepatitis or "blackhead," make the control of the cecal worm of economic importance.

The reports of Harwood, Jerstad and Swanson,[5] and of others,[6,7,8] who successfully used phenothiazine in removing nematode parasites from swine, sheep, and other animals, suggested to the authors the possibility that this drug might be of value as a poultry anthelmintic.

Experimental

A farm flock of heavily parasitized two- to four-year-old S. C. White Leghorn chickens was purchased. These birds were in from poor to fair condition; autopsies of representative birds revealed heavy infestations with cecal worms, moderate infestations with round worms, and variable degrees of infestation with tapeworms. Some of the birds showed evidence of tuberculosis, pullorum disease and leucosis.

The birds were confined in individual, wire-bottomed cages, and the droppings were collected at 24-hour intervals. The fine material was washed from the droppings in a 40-mesh screen and the nematodes collected and counted against a black background, using a brilliant light. Viability was determined by placing the cecal worms in water at 45° C. and observing them for motility. At the conclusion of each trial, autopsies were performed and the intestinal contents examined for the presence of parasites by the same method. Identical results were obtained with two lots of phenothiazine; one, a granular powder from the Eastman Kodak Company, and the other, a very fine powder from the E. I. Du Pont Company.

The results of single doses of phenothiazine are shown in Table I. Dosages between 0.05 gm and 1.0 gm were highly effective in killing and removing the cecal worms. Except for birds 386 and 265, which consumed only a small amount of the feed with which the drug had been mixed, the administration of the drug in capsules had only a slight advantage.

The results of repeated doses of phenothiazine are shown in Table II. Slightly better results were obtained by the administration of repeated doses of phenothiazine than by single doses.

An attempt was made to determine the toxic dose of phenothiazine by administer-

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[1] Published as scientific paper No. 461, Washington Agricultural Experiment Station, State College of Washington; contribution from the Division of Veterinary Science, Pullman, Washington

[2] Gildow, E. M., C. E. Lampman, P. Moore, and Glen C. Holm, 1939. Prevention and control of poultry diseases. University of Idaho, Ext. Bull. No. 119.

[3] Graham, Robert, J. P. Torrey, J. D. Mizelle and Viola M. Michael, 1937. Internal parasites of poultry, University of Illinois, College of Agr. Cir. 469, pp. 1-50.

[4] Graybill, H. W., and Theobald Smith, 1920. Production of fatal blackhead in turkeys by feeding embryonated eggs of *Heterakis papillosa*. *Jour. Exp. Med.*, XXXI, pp. 647-655.

[5] Harwood, Paul D., Arthur C. Jerstad and Leonard E. Swanson, 1938. The efficacy of phenothiazine for the removal of ascarids and nodular worms from swine. *Jnl. Parasitol.*, 24:6, suppl. pp. 16-17.

[6] Harwood, Paul D., Robert T. Habermann, and A. C. Jerstad, 1939. Efficacy of commercial phenothiazine in the removal of roundworms from sheep. *Vet. Med.* 34:7, pp. 440-443.

[7] Gordon, H. McL., 1939. A preliminary note on the chemotherapy of oesophagostomiasis of sheep with special reference to the efficiency of phenothiazine. *Jour. Counc. Sci. and Ind. Res.*, Australia, XII, pp. 203-206.

[8] Roberts, F. H. S., 1939. A preliminary note on the treatment of oesophagostomiasis in sheep with phenothiazine. *Jour. Counc. Sci. and Ind. Res.*, Australia, XII, p. 208.

TABLE I. EFFICIENCY OF A SINGLE DOSE OF PHENOTHIAZINE IN KILLNG AND EXPELLING HETERAKIS GALLINAE

Bird No.	Weight (grams)	Dose (grams)	Heterakids on successive days 1	2	3	Total expelled	Remaining at autopsy Live	Dead	Per cent efficiency Expelled	Killed
274	1588	1.0 in capsule	400	64*		464	1	5	98.7	99.7
290	1446	1.0 in capsule	256	48*		304	0	1	99.7	100.0
293	1644	0.5 in capsule	140*			140	0	14	90.9	100.0
243	1446	0.5 in capsule	160	0*		160	0	0	100.0	100.0
265	1191	0.5 in feed	64*			64	78	702	7.6	90.7
268	1644	0.1 in capsule	432*			432	0	8	98.2	100.0
338	1644	0.05 in capsule	112	0	0*	112	0	0	100.0	100.0
318	2410	0.05 in capsule	140	12	4*	156	2	14	91.3	98.9
350	2098	0.05 in capsule	35	4	0*	39	0	8	83.0	100.0
386	1004	0.05 in feed	60	0	0*	60	42	0	58.8	58.8
307	1646	0.05 in feed	40	0	0*	40	0	15	88.9	100.0
369	2268	0.05 in feed	60	25	0*	85	0	1	98.8	100.0

* Autopsy performed on day indicated.

ing enormous amounts to experimental chickens. In the first group, four 90-day-old S. C. White Leghorn chickens weighing 907, 907, 1134, and 964 grams were each given 10, 10, 5, and 5 grams respectively of phenothiazine in hard gelatine capsules. Observations were made at frequent intervals, but aside from a slight declination of temperature about six hours after administration, no harmful effect was noted. Appetite and thirst were seemingly unaffected.

TABLE II. EFFICIENCY OF REPEATED DOSES OF PHENOTHIAZINE IN KILLING AND EXPELLING HETERAKIS GALLINAE

Bird No.	Weight (grams)	Dose (grams)	Days given	Total given (grams)	Heterakids expelled in droppings on successive days 1	2	3	0	5	6	7	Total expelled	Remaining at autopsy Live	Dead	Per cent efficiency Expelled	Dead
288	1474	1.0 in cap.	2	2.0	30	240*						270	0	150	64.3	100.0
276	1871	1.0 in cap.	6	6.0	15	0	0	0	0*			15	0	0	100.0	100.0
206	1559	0.5 in cap.	2	1.0	160	0*						160	0	5	97.0	100.0
209	1502	0.5 in cap.	6	3.0	0	650	372	0	4	0*		1026	0	1	99.9	100.0
251	1446	0.1 in cap.	2	0.2	160	12*						172	0	4	97.7	100.0
280	1616	0.1 in cap.	7	0.7	120	0	6	0	0	0	2*	128	0	0	100.0	100.0
270	1616	0.1 in cap.	4	0.4	568	136	272	24*				1000	0	1	99.9	100.0
371	2013	0.05 in cap.	2	0.1	0	0	0*					0	0	52	0.0	100.0
320	1503	0.05 in cap.	2	0.1	150	48	2*					200	0	0	100.0	100.0
313	2155	0.05 in cap.	2	0.1	320	68	0*					388	0	0	100.0	100.0
404	1446	0.5 in feed	2	1.0	560	16*						576	0	1	99.8	100.0
281	1502	0.5 in feed	3	1.5	280	24	0*					304	0	0	100.0	100.0
253	1557	0.5 in feed	3	1.5	48	32	0*					80	0	0	100.0	100.0
263	1503	0.5 in feed	3	1.5	320	24	0*					344	0	63	84.6	100.0

* Autopsy performed on day indicated.

In a later trial five adult S.C. White Leghorn hens weighing 1231, 1316, 1443, 1414, and 1402 grams were given 5, 10, 15, 20, and 25 grams respectively of phenothiazine in hard gelatine capsules. Frequent observations were made. The dye made its appearance in the droppings one and one-half hours following administration. Fifteen hours after administration the birds were apparently unaffected by the drug except for a slight softening of the droppings. It is interesting to note that the birds that received 15, 20, and 25 grams of the drug voided some of the drug seemingly unaltered in the droppings. Autopsy of the bird that received 25gm of phenothiazine revealed a severe infestation of heterakids, remaining apparently unaffected in the ceca. This probably was due to sufficient stimulation of the intestinal tract having been produced by the massive dose of the drug so that it was not taken into the ceca. Following autopsy, three days after the administration of this massive dose, this bird was cooked and eaten by its former owner, who reported the flavor of the meat unaffected.

To determine if the drug had a noticeable effect on egg production, two pens of S. C. White Leghorn chickens were given individual capsules. The birds in one pen received 0.5gm of phenothiazine; the birds in the other pen received empty capsules. Records of egg production made before and after the administration of the drug showed no appreciable difference.

Discussion

The data presented reveal that phenothiazine, when used in moderate doses, is a very effective anthelmintic for the removal of cecal worms from chickens. Considerable latitude is possible in the determination of

the proper therapeutic dose. It is the opinion of the authors that between 0.05 and 0.5 grams per bird is a reasonable amount to recommend. Enormous doses, although harmless to the birds, have little if any effect on the cecal worm. Repeated doses seem to

Blackhead (enterohepatis) is a common disease of turkeys. This bird exhibits symptoms characteristic of the last stages of the disease.

be slightly more efficacious than single doses. It is apparent that birds receiving individual capsule medication are more effectively and surely treated. However, the margin of safety with this drug is so great that flock medication in the ration for two or three days appears to be practical. Some of the birds were fasted before administration of the drug; however, there was no noticeable difference in heterakid expulsion or killing power. The use of phenothiazine caused a slight softening of the droppings for approximately 24 to 48 hours. No evidence of enteritis either in the small intestine or ceca was noted on autopsy. Appetite was seemingly unaffected or perhaps slightly increased. Water consumption increased slightly 24 hours following treatment but was not outstanding. Egg production continued normally even in those birds receiving enormous doses.

Although the most desirable dosage of phenothiazine has not as yet been definitely determined, the apparent merits of the drug and its seeming lack of toxicity suggest it as the anthelmintic of choice against *Heterakis gallinae*. Further work is planned relative to the proper dose, as experimental evidence was obtained that larger doses are effective against ascarids. The cost of phenothiazine is low.

Summary

1. *Heterakis gallinae* is a common parasite of chickens that serves as a vector for *Histomonas meleagridis*, the causative organism of enterohepatitis. In addition large infestations cause severe typhlitis.

2. Heretofore, no entirely satisfactory anthelmintic for this parasite has been reported. Those previously recommended either are difficult to administer or are low in effectiveness.

3. Phenothiazine was administered to S. C. White Leghorn chickens suffering from a natural heavy infestation of cecal worms. This drug was given either in the feed or in hard gelatine capsules. Single and repeated doses were tried.

4. Between 0.05 and 0.5 grams was found to be a satisfactory individual dose.

5. Repeated doses and administration of the drug in individual capsules were found to be slightly more satisfactory, although flock medication appears to be practical.

6. The average effectiveness was between 95% to 100%, both from the standpoint of cecal worms expelled and killed.

7. Enormous doses, up to 500 times the smallest amount therapeutically effective, had no apparent harmful effect on the birds; such massive doses also had no anti-heterakid effect.

8. Individal capsule medication of 0.5gm had no appreciable effect on a flock in egg production.

9. Neither massive nor therapeutic doses had any effect upon the flavor of the meat.

10. The administration of phenothiazine was not followed by enteritis or other digestive disturbances, except for a slight softening of the feces 24 hours following treatment.

11. The use of phenothiazine as an anthelmintic for the removal of heterakids is recommended, even though the most desirable dosage has not been definitely determined.

Coccidiosis of Domesticated Birds

With Special Reference to the Common Fowl

By E. R. BECKER, Ames, Iowa,
Iowa State College

COCCIDIOSIS is the state of infection with any species of the Coccidia, a large taxonomic division of the animal microbes or Protozoa. These microorganisms are parasitic for at least the major part of their life cycles, and are tissue-dwellers. The coccidia exhibit extensive diversity in development, so that they have been subdivided into suborders, families, subfamilies, genera, and species. Thus the term coccidiosis covers not a particular type of disorder with restricted etiology, but rather myriad types of infection. The hosts concerned represent such diverse animal groups as worms, centipedes, insects, mollusks, fishes, frogs, snakes, birds, and mammals, including man. The scope of the present discussion can be limited to a relatively few types of coccidiosis, however, because of one remarkable fact: The species exhibit marked, and almost exclusive, host-preferences; or, conversely, they do not distribute themselves promiscuously among the higher animals.[1] Thus, in discussing coccidiosis of the common fowl, attention can be focused almost entirely on the form and behavior of six or seven species of etiologic agents and the distinctive features of the impact of each on the host.

The species of coccidia capable of dwelling in the chicken are all members of the genus Eimeria; that is, the oocyst encloses four spores, each containing two sporozoites, in the infective stage. In fact, all birds of the order Galliformes (chicken, turkey, guinea-fowl, pheasant, quail, etc.) are parasitized with coccidia of the genus Eimeria, while the Passeriformes (sparrow, robin, blackbird, etc.) harbor the genus Isospora, characterized by oocysts containing two spores, each provided with four sporozoites when completely developed. Boughton's recently published analysis[2] of the distribution of Eimeria and Isospora among these and other orders of birds shows most convincingly that the distribution of the two genera conforms to host type. Consequently, one would not expect to find sparrows and other wild birds frequenting barnyards serving as reservoirs for the chicken coccidia. There are also a number of experiments on record which demonstrate that sparrow coccidia of the genus Isospora do not develop in chickens. In other words, avian coccidia are host-specific.

Life Cycle

A second fundamental concept for an understanding of poultry coccidiosis is the life cycle. The reader is referred to more general works, such as Becker[1] for details and figures of the sequence of transformations that a coccidium undergoes preceding and during an infection, but there are certain salient features of the development that should be reemphasized. In the first place, the portal of entry is the digestive tract. Ordinarily sporulated oocysts gain admission to the host with food and drink. Under the combined action of digestive juices and favorable temperature, excystation takes place. The process involves the release of eight sporozoites, in reality the infective stages, from the spores and oocyst. Pratt[3], working with *Eimeria tenella* in chickens, found excystation occurring in the crop, sometimes within five minutes after ingestion of the oocysts, although it could occur also further down in the intestine. He observed that spores were liberated from the oocysts before the sporozoites broke from the spore, and that the spores came out through the side of the oocyst wall.

The first penetration of the host tissue is by the sporozoites, which make their way actively into the cells of the intestinal epi-

[1] Becker, E. R., 1934. Coccidia and Coccidiosis of Domesticated, Game, and Laboratory Animals and of Man. Ames, Iowa.

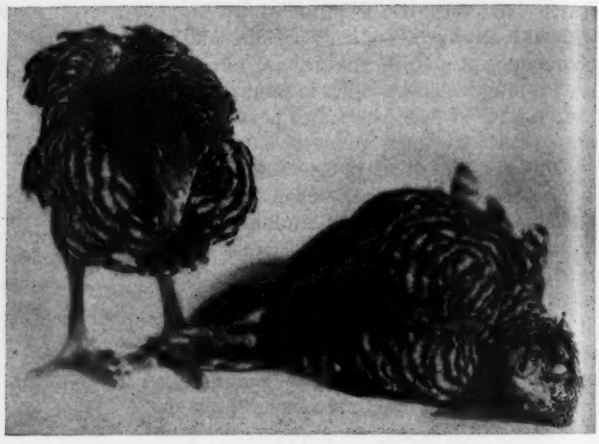
[2] Boughton, D. C., 1937. Notes on avian coccidiosis. *The Auk*, 54, pp. 500-509.

[3] Pratt, I., 1937. Excystation of the coccidia, *Eimeria tenella*. *Jour. Parasit.* 23, pp. 426-427.

thelium. The site of election varies according to species. *Eimeria tenella* localizes in the ceca and immediately adjacent regions of the small and large intestines. The initial attack of *Eimeria necatrix* is on the wall of the small intestine, being most intense in the middle region, though later stages develop in the ceca. *Eimeria mitis, E. acervulina,* and *E. praecox* concentrate in the upper part of the small intestine. *Eimeria maxima* develops throughout the small intestine, but by far the heaviest concentration is in the mid-region and just back of it.

The sporozoite accommodates itself to life within an appropriate epithelial cell, grows into a schizont. A characteristic of a schizont is that it is predestined to undergo schizogony, *i.e.,* undergo a series of nuclear divisions and eventually split up into a number of nucleated fragments, or merozoites, which escape from the depleted host cell. The merozoites in turn enter other cells, and the process of schizogony is repeated a number of times. Whether this phase of the cycle is self-limited is a moot point, but after several or more repetitions there appear merozoites of a new generation which, when they enter new cells, become transformed into sexual individuals—male and female. A process of fertilization ensues, the product of which is an oocyst—in reality a zygote surrounded by a wall.

Oocysts are shed from the intestinal lining, usually in enormous numbers, and appear in the droppings of the birds. These freshly passed oocysts are not the infective stages, however. In the presence of moisture, oxygen, and favorable temperature, the zygote undergoes a transformation inside the wall. First, it becomes divided into four rounded or oval bodies, each provided with a nucleus, that secrete walls, or sporocysts, about themselves, so that they have been called spores. From the protoplasm inside each spore are carved out two banana-shaped sporozoites, each with a nucleus. Thus is completed the development of the sporulated oocysts of the genus Eimeria.



Coccidiosis in Chickens.—*Photo courtesy of Robert Graham*

The time required for an oocyst to sporulate is called the sporulation time, and varies somewhat according to species.

Species Parasitic in Chickens

There are at least six, and possibly seven species of coccidia known to infect chickens. Tyzzer and his collaborators[4, 5] have given six species extensive treatment elsewhere, so that here it is necessary to review only the salient features of each. For another excellent paper on the subject and excellent photomicrographs of the coccidia, the reader is referred to Bulletin 358 of the Oregon Agricultural Experiment Station.[6]

Eimeria tenella is the cause of so-called cecal or bloody coccidiosis of chicks. Involvement of the ceca rather than of the intestine is one of its characteristic features. The severity of this type of coccidiosis is attributable to the second generation schizont, which causes infected epithelial cells to increase tremendously in size and assume

[4] Tyzzer, E. E., 1929. Coccidiosis in gallinaceous birds. *Am. Jour. Hyg.* 10, pp. 269-383.

[5] Tyzzer, E. E., H. Theiler, and E. E. Jones, 1932. Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria of the chicken. *Am. Jour. Hyg.* 15, pp. 319-393.

[6] Johnson, W. T., 1938. Coccidiosis of the chicken with special reference to species. Ore. Agr. Exper. Sta. Bul. 358.

a migratory habit. Through pressure or otherwise there is produced sufficient degeneration of the blood vessels and surrounding tissues to result in bleeding into the ceca, and the well-known copious bloody discharges from the ceca. The discharges usually commence to appear bloody sometime before the end of the fifth day after infection, but a certain wateriness of the droppings is sometimes noted much earlier.

Oocysts appear in the droppings commencing on the seventh day. Their discharge continues more or less continuously over a considreable period. Herrick, Ott, and Holmes[1] found them in the droppings of chickens up to 7½ months after the infection date. Their study showed that following infection the oocysts are enmeshed in the tissue of the ceca where they remain viable for at least 7½ months. Sporulation of oocysts of *E. tenella* requires a little less than 48 hours.

Eimeria necatrix is another of the so-called pathogenic coccidia of the chicken. This species attacks the small intestine, with the maximum involvement near the middle. The second generation schizonts are similar in form and behavior to those of *E. tenella*, and like them produce the pathogenic effects. On the fourth and fifth days aggregations of these schizonts appear as small whitish opacities. Later, punctate hemorrhages appear in the center of the whitish areas, and may become so extensive as to obscure them altogether. As Tyzzer and collaborators state, "The unopened intestine thus presents a spotted appearance, the small whitish areas being intermingled with rounded, bright or dull red blotches of various sizes while transversely extending reddish streaks represent hemorrhages along the superficial vessels." There is profuse hemorrhage into the intestinal lumen.

Necatrix disease may be of two types—acute or chronic. The former may result in the death of the bird five to seven days after infection, while in the latter case the bird may linger on for a long time with a wasting illness. During the acute attack blood may be observed in the droppings.

E. necatrix is unique among fowl coccidia in that, while the first two generations of schizonts develop in the small intestine, the merozoites generated by the second generation schizonts migrate to the ceca where they invade the epithelium and develop, some into further generations of schizonts and some directly into oocysts.

Oocysts appear in the droppings on the seventh day after infection, and ordinarily require two days to sporulate. Tyzzer found that far more of them are produced in light infections than in heavy ones. An infected bird may discharge oocysts over a prolonged period. The writer believes *E. necatrix* infection to be rare since he has never seen it.

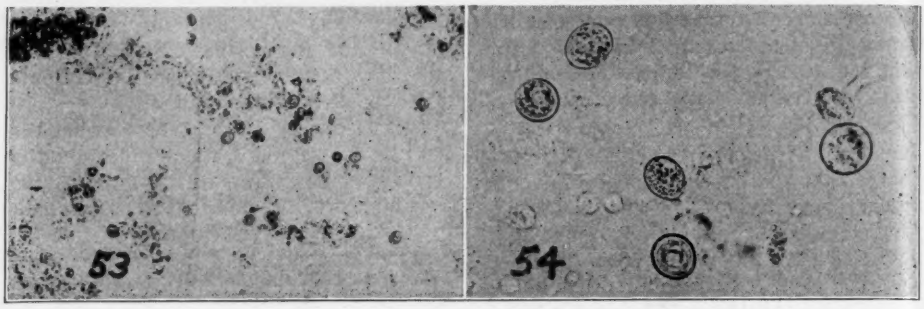
Eimeria maxima is the third of the definitely pathogenic species in the chicken, though far less lethal than the other two. Clinically, the recognition signs are dilation of the small intestine and thickening of the wall. The serous surface may show faint hemorrhages. The content is not bloody, but takes the form of viscid mucus, grayish, brownish, or pinkish in color. In some instances the portion of the feces from the small intestine may show flecks of blood. Infections sometimes terminate fatally, though the writer once infected a lot of 30 three-week-old birds without losses, or even serious symptoms.

Oocysts appear in the droppings on the sixth day after infection and continue to do so for only a few days. After oocyst elimination ceases the bird usually possesses a high degree of immunity to reinfection, and is definitely protected from recurring clinical symptoms. The oocysts of this species are quite characteristic, being the largest of all occurring in chickens (about 29.3 microns by 22.6 microns on an average), and having a slightly roughened wall.

Eimeria acervulina is, fortunately, not a severe pathogen, though it is perhaps the commonest of all the poultry coccidia. It is characterized clinically by numerous gray or whitish patches in the upper half of the small intestine, visible through the serous surface. These patches are caused by forming oocysts.

[1] Herrick, C. A., G. L. Ott, and C. E. Holmes, 1936. The chicken as a carrier of the oocysts of the coccidia, *Eimeria tenella*. *Poul. Sci.* 15, pp. 322-325.

That the species is not very pathogenic, is quite likely a fair statement of the situation. The writer once inoculated 60 white leghorn chicks with 300,000 sporulated oocysts each. The droppings which fell through hardware cloth onto newspapers were definitely more watery on the third day under the infected birds than under the controls, and the appetite was undoubtedly impaired for two or three days, but there were no losses. Oocysts are eliminated in tremendous numbers, commencing the fourth day.



Oocysts of *Eimeria tenella*, x100 (left) and x400 (right).—*Photos by Benbrook*

Eimeria mitis grows in the small intestine throughout its entire length, but is most concentrated in the upper half. It is definitely not a serious pathogen. Using the test alluded to in the discussion of *E. acervulina*, that is, size of the wet rings around the portions of droppings of small intestinal origin, the writer has not been able to ascribe any edema of the small intestine to this species. The oocysts are small, with a tendency to the spherical, and usually are not abundant in the droppings.

Eimeria praecox develops in the upper third of the small intestine. This species is probably no more pathogenic than *E. mitis*, as the author has verified in an extensive test. Oocysts, however, are passed in superabundance, commencing on the fourth day.

Eimeria hagani was recently established as a new species by Levine[8]. It will be described here in detail since it does not appear in either Becker[1] or Johnson[6]. The infection is marked post mortem by numerous round hemorrhagic spots of the size of a pin-head visible through the serosa in the duodenum and the upper half of the remainder of the small intestine, and comparatively few such lesions in the lower half. The oocysts, which develop mostly in the affected regions mentioned, commence to be discharged towards the end of the sixth day and appear in great number on the seventh day. Chickens immunized to *E. acervulina*, *E. præcox*, *E. mitis*, and *E. maxima*, respectively, can be infected with *E. hagani*, thus demonstrating the specificity of that form. Birds autopsied on the sixth day of the infection exhibit "a severe catarrhal inflammation of the duodenum and anterior half of the small intestine."

The average size of sporulated oocysts is 19.1 by 17.6 microns. Sporulation requires 24 to 48 hours.

Control

Since, so far as is known, poultry coccidia are in general host-specific, segregation of chickens from ducks, geese, turkeys, and guinea-fowl is not indicated, so far as coccidiosis control is concerned. The same holds for wild birds as true biological carriers. This is not to say that sparrows, pigeons, blackbirds, and robins cannot disseminate oocysts mechanically, either by carrying them on the feet or by ingesting them in food picked up on infested premises and evacuated on "clean" grounds. There might conceivably be situations where in the interest of protecting one's flock from coccidiosis (and other diseases) it would be

[8] Levine, P. P., 1938. *Eimeria hagani* n. sp. (Protozoa: Eimeriidæ) a new coccidium of the chicken. *Cor. Vet.* 28, pp. 263-266.

wise to enclose the poultry run overhead with wire netting to preclude the visitations of sparrows and the like, but in so doing it should be kept in mind that it is a neighboring flock which is the primary source of infection, not the visiting birds.

Other methods of mechanical dissemination are flies, the hands and feet of attendants, dogs, etc. During the fly season it is possible to reduce fly hazards by screening brooder houses and using fly sprays. It is often impracticable for the attendent thoroughly to wash his hands and clean his shoes when going from one pen to another, but he can be careful not to disseminate mass infection. Up to a certain point the severity of coccidia infections depends on the number of oocysts ingested, so the value of preventing the dissemination of oocysts as far as possible is evident.

There are still other sanitary measures that have been employed with success. Cleaning the brooder houses and runs every day is a laborious process, but it does accomplish the removal of infection, since it ordinarily requires but two or three days for the oocysts of the most pathogenic forms to sporulate. Automatic removal of feces, and hence of sources of infection, has been accomplished by raising chickens on hardware cloth and later on floors of mesh made from cold rolled rods, or other grilled surface. Then there is the "deep litter method," recently tested experimentally by Boughton.[9] It is based on the hypothesis that the opportunity for ingestion of sporulated oocysts is limited by keeping the birds on deep litter, stirred daily. The chicks are put on the litter at starting, and the litter remains unchanged until preparations are made for the next brood. Results of the practice are conflicting, but Boughton's study indicates that drying of the feces in shavings or sawdust does reduce the percentage of oocysts sporulating, while the results with wet litter varies according to the degree of fouling. Wet, clean litter provides highly favorable conditions for sporulation, while the reverse is true of wet, highly fouled litter. Presumably some of the benefits derived from deep litter, if there are any, are to be attributed to dilution of the sources of infection. Boughton promises to continue his study in relation to coccidiosis control.

The feeding of sulphur in the ration has been advanced recently as a practice having promising possibilities for the control of *Eimeria tenella* infection. According to a mimeographed information sheet which the writer received from the Freeport Sulphur Company in 1937, B. F. Jarvis in the fall of 1934 disclosed to that company his "Two Point Method" of controlling coccidiosis with sulphur. The two "points" were as follows: (1) applying sulphur to the litter "through the medium of the droppings had value in preventing putrefaction and in maintaining the litter in a dry, pulverulent state" (2) feeding a 10% sulphur mash one full day each week resulted in broiler flocks remaining free of clinical evidence of coccidiosis for the full 12-week test period. Chickens on infested ranges, however, were not fully protected by this method.

The work of Herrick and Holmes,[10a] however, has received the most prominence. These workers carried out two sets of experiments, one with sulphur fed from hatching until infection at the age of three weeks, another with sulphur fed for six days prior to infection. In the first case the ingredient was mixed in the ration at the 2%, 5%, and 10% levels; in the second, at the 10% and 20% levels. Since mortality from experimental *E. tenella* infection was strikingly reduced, in both tests, the feeding of sulphur seemed to offer considerable promise. The group of chicks fed sulphur from hatching, however, developed irritation of the cloaca and their growth rate was markedly retarded. The group fed sulphur for only six days prior to infection did not show these adverse effects. Herrick and Holmes[10] later reported that continued feeding of sulphur at the 5% or higher level resulted in rickets when cod-liver oil

[9] Boughton, D. C., 1939. Studies on the control of poultry coccidiosis. I. The sporulation of oocysts in various types of litter. Reprint from the Bul. Univ. Ga., 39:8.

[10a] Herrick, C. A., and C. E. Holmes, 1936. Effects of sulphur on coccidiosis in chickens. *Vet. Med.*, 31:9, pp. 390-391.

[10] Herrick, C. A., and C. E. Holmes, 1937. Sulphur helps to prevent coccidiosis. Wisc. Agric. Exper. Sta. Bull. 438, pp. 36-38.

was used as the source of vitamin D. Holmes, Deobald and Herrick[11] followed up with a number of comprehensive tests, concluding: "If chicks are fed 2% or more of granular sulphur, wettable sulphur, or flowers of sulphur, and are entirely dependent upon cod-liver oil of the quality tested in these trials, rickets is quite likely to develop." Probably the correct estimation of the status of sulphur is that it has merit in coccidiosis control, but ill effects otherwise that have accompanied its feeding to date do not warrant its recommendation in poultry-raising practice. It would be most encouraging if a method of its use could be devised which would retain its merits and eliminate its shortcomings.

The relation of the amount of calcium carbonate in the ration to coccidiosis also has a bearing on control. Holmes, Herrick, and Ott[12] showed quite clearly that when this ingredient was fed at the 6% level mortality was considerably higher than when it was either not fed or held at the 3% level. The feeding of calcium carbonate to growing chicks at high levels is a questionable practice anyhow, since Halpin[13] has shown that 2% is sufficient in the usual chick ration.

The value of at least 40% of dried milk, skim milk or buttermilk in the ration for the control of cecal coccidiosis has received much attention since it was first recommended by Beach and Corl[14] and Beach and Davis.[15] Following is the formula for a recommended mash[16]: dry skim milk or buttermilk, 40 lbs.; wheat bran, 10 lbs.; yellow cornmeal, 30 lbs.; ground barley, 20 lbs. Possibly ground whole oats could be substituted for the barley[17]. It is recommended that this mash be offered as soon as bloody droppings are first noted in the flock, and no other stuffs fed except an amount of grain equal to one-third to one-half of the weight of the mash consumed. Fresh water, of course, should be available at all times. This regimen is practiced for one or two weeks only, by which time the outbreak is supposed to be checked, and then the usual ration is gradually resumed. The merit of the system has been ascribed to various factors: (1) the creation of an acid condition in the ceca that is unfavorable to the coccidia; (2) the ability of the 40% dry milk mash to stimulate growth; (3) flushing parasites from the ceca, thus reducing the effect of mass parasite depredations; (4) "protein death" for the coccidia (quoting a salesman for a feed concern).

The idea of incorporating 40 parts of dry milk in each 100 parts of mash has been used with good, indifferent, and bad results, according to many reports in the literature. Furthermore, the flushing effect of such a mash results in both high water consumption and elimination, making the floor or litter wet and thus a favorable medium for the sporulation of oocysts. An inspection of the rations fed by most of the workers who have been unsuccessful with the high dry milk mash shows that they did not follow the formula exactly.

Becker and Wilcke[17] made a test with four rations, among which were three of immediate interest. Ration II was of the ordinary type, containing ground yellow corn, ground oats, bran, wheat middlings, soy bean oil meal, alfalfa, meat scrap, fish meal, oyster shell, salt, cod-liver oil, and 10 parts of dried buttermilk. Ration III had all of these ingredients except that soy bean oil meal was omitted and dried buttermilk raised to the 40% level. Ration IV consisted of ground yellow corn, 30 lbs., ground oats, 20 lbs., wheat bran, 10 lbs., dried buttermilk, 40 lbs., cod-liver oil, 1 lb. Thus Ration IV was similar to the Beach and Freeborn (or "California") ration described above, except that ground oats was substituted for ground barley and cod-liver oil was added. The chicks were started on a selected grow-

[11] Holmes, C. E., H. J. Deobald, and C. A. Herrick, 1938. Sulphur and rickets. *Poul. Sci.* 17, pp. 136-142.
[12] Holmes, C. E., C. A. Herrick, and G. L. Ott, 1937. Studies in coccidiosis in chickens: Calcium carbonate additions and coccidia. *Poul. Sci.* 16, pp. 335-339.
[13] Halpin, J. G., 1926. Minerals influence rate of growth of chicks. Wisc. Agric. Exper. Stat. Bul. 388, p. 111.
[14] Beach, J. R., and J. C. Corl, 1925. Studies in the control of avian coccidiosis. *Poul. Sci.* 4, pp. 83-93.
[15] Beach, J. R., and D. E. Davis, 1925. The influence of feeding lactose or dry skim milk on artificial infection of chicks with *Eimeria avium*. *Hilgardia* 1, pp. 167-181.
[16] Beach, J. R., and S. B. Freeborn, 1933. Cal. Agric. Ext. Ser. Circ. No. 8, Revised, pp. 47-57.
[17] Becker, E. R., and H. L. Wilcke, 1938. The influence of dried buttermilk in rations on fatality with coccidiosis in chicks. *Poul. Sci.* 17, 405-407.

ing ration for 16 days, then divided into three lots and fed the test rations thereafter. On the tenth day each chick was infected with a heavy dose of the sporulated oocysts of *Eimeria tenella*. There were 39 fatalities among 54 chicks on Ration II; 31 among 52 chicks on Ration III; and but 13 fatalities among 55 chicks on Ration IV. The difference between the fatalities on Ration II and Ration IV, and that between Ration III and Ration IV, were statistically significant. The experiment proved that Ration IV, with its 40% dried milk and simple formula, was an aid in reducing fatality from cecal coccidiosis when it was fed for almost 10 days before the date of infection. The experiment was later repeated (unpublished data) with the same general results, although the difference between the effects of Ration III and Ration IV was not so large. It should be noted that the effect of commencing to feed Ration IV after the chicks had been infected was not tested, and that Ration IV did not prevent fatalities, for almost a quarter of the chicks eating it died.

Along with the groups of chicks on the three rations was another on Ration I, essentially like Ration II except that it contained much more soy bean oil meal and no dried buttermilk. There were but 16 fatalities among 53 chicks eating this ration. The differences between the fatalities on Rations I and II, and I and III, were statistically significant, but not that between Rations I and IV. This experiment, too, has been repeated with essentially similar results. Here then is a curious situation, with a ration made up to 40% with dry buttermilk effecting a reduction in fatalities and a ration without dry milk affording a similar result. The average gain in weight per chick, however, was about a gram a day more on the high dry buttermilk ration than on the ration without milk.

Other feeding experiments[18] in which dry milk was omitted from the ration have usually resulted in a number of the chicks developing "curly toe" paralysis, which condition certain workers have shown to be attributable to lack of riboflavin. It has been found by the writer, however, that as little as 1% dry skim milk or dry buttermilk will ordinarily prevent this condition. Since the combination of dry skim milk at the 5% level and wheat middlings in the mash at the 30% level seemed to favor the development of severe cecal coccidiosis, a ration was planned in which both would be limited to lower levels and a greater variety of grain would be included. The result was the following: ground yellow corn, 40; ground hulled oats, 10; finely ground whole oats, 5.5; ground whole barley, 5; ground whole wheat, 5; wheat bran, 5; wheat middlings (gray shorts, 16.5% protein), 5; soy bean oil meal (expeller process, high temperature), 10.5; meat scraps, 4; fish meal, 2; fine oyster shell, 2; salt, 1; dry skim milk, 2; alfalfa meal, 3; cod-liver oil, 1. The results of the test were very satisfactory, with losses reduced to one-third of those on the control ration. While the writer has not recommended the ration to practical poultry raisers, he has received oral reports from certain commercial concerns which have given it a number of large scale tests, and found that it afforded both satisfactory growth and a high degree of protection from severe cecal coccidiosis. It is certainly worthy of further testing by poultry husbandrymen.

This, then, represents one of the attempts to mitigate the severity of cecal coccidiosis by adjusting the diet. More recent work[19] has shown that certain kinds of fish meal in the ration may also be unfavorable for the course of the infection.

There have been many other suggestions for controlling coccidiosis, and doubtless some of them have merit. Among them chemical treatment of the litter[20] and immunization of birds with heated oocysts[21] are deserving of further consideration.

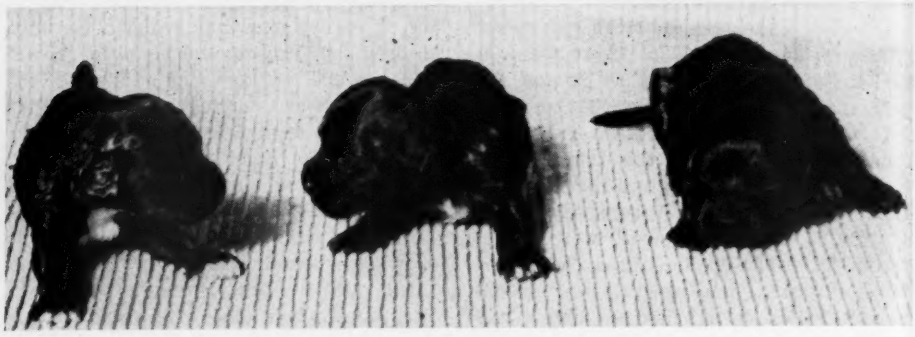
[18] Becker, E. R., and P. H. Waters, 1938. The influence of the ration on mortality from cecal coccidiosis in chicks. *Ia. St. Col. Jour. Sci.* 12, pp. 404-414.

[19] Becker, E. R., and P. H. Waters, 1939. Dried skim milk and other supplements in the ration during cecal coccidiosis of chicks. *Proc. Soc. Exper. Biol. and Med.* 40, pp. 439-442.

[20] Andrews, J., 1933. Poultry coccidiosis control by the chemical treatment of litter. *Poul. Sci.* 12, pp. 133-140.

[21] Jankiewicz, H. A., and R. H. Scofield, 1934. The administration of heated oocysts of *Eimeria tenella* as a means of establishing resistance and immunity to cecal coccidiosis. *J. A. V. M. A.* 84, pp. 507-526.

Permanent Distemper Immunization



HEALTHY PUPPIES

These puppies will in due course be vaccinated against distemper, probably by the Laidlaw-Dunkin method. This should protect them—one reason why it may not is explained in this article

SINCE the introduction of the Laidlaw-Dunkin method of canine distemper immunization, sufficient time has elapsed to evaluate its efficacy to some degree. There never has been much uncertainty of the effectiveness of the method under experimental conditions; there its value has been established beyond a doubt. One cannot say as much for field results, beneficial as they have been.

In attempting to evaluate the effectiveness of distemper immunization one constantly runs into the difficulty that there are no controls with which to compare the treated dogs; also, thousands of dogs which are immunized are never exposed. "My dog never caught distemper—had him immunized," is a familiar phrase heard among dog breeders.

But there are also many dissatisfied clients who have had dogs immunized which later contracted distemper and died. To such, the treatment is a complete waste of money. These cases are not exceptional. When many owners of large kennels have tried the treatment and found it inadequate, it gives us something to consider.

When one asks such owners if, following the introduction of the dose of virus, they noted any reaction in their dogs, quite often the reply is negative. In their conclusions Laidlaw and Dunkin state that a dog to be solidly immune must show some reaction.

The author knows personally owners of large kennels, who themselves are careful observers, and who engage competent kennel men or women who are also careful observers. Many of them have related experiences worth careful study. Some have had experiences with the Laidlaw-Dunkin method which pleased them; others have declared it useless as administered to their dogs. More have had happy experiences one time and met with disappointments the next.

Some of these men and their veterinarians have observed what appeared to be solid immunity induced in dogs when they were treated with two doses of vaccine and no virus. But they could not be sure in all cases because of a lack of real controls. As an example, all of the supposedly susceptible dogs in one kennel were inoculated with vaccine and two weeks later inoculated again. The owner was then advised to take her dogs to a dog show so they might become exposed and develop solid immunity. This was done, no dog became sick, and the proprietor believes they are all solidly immune. But even here there is no certainty.

Another kennel owner insists upon three doses of vaccine and no virus, because of an experience during which he lost many dogs when the virus was administered. Since using three doses of vaccine, no case of distemper has appeared in the kennels

By Vaccine Alone

By LEON F. WHITNEY,
Orange, Connecticut

even though the owner is certain there has been opportunity for exposure. Again, this is only a matter of conjecture.

In these and similar ways, veterinarians and kennel owners have had their ideas apparently verified, but usually in the absence of controls. Again, there are many instances where vaccine alone has been used on all the dogs in a kennel, and when an epizootic sweeps through the kennel only those newcomers and puppies born after the last lot were immunized have become sick.

Then there are those who have come to the conclusion that because no reaction follows the administration of virus, this is *prima facie* evidence that it is dead. The following data will furnish at least a partial answer to these problems.

Evaluation of Commercial Dried Virus

After one experience in which a number of dogs were given vaccine followed by virus, and no reaction occurred, it was decided to test samples of the dried virus sold to veterinarians. It was imperative to know something about this material before undertaking to estimate the value of, and the reason for apparent failure of, the field procedure. Puppies were segregated into groups. Several groups were given injections in one of the laboratories of the Yale University Medical School, where the author was then studying bacteriology. Others were injected in his own laboratory.

The virus used carried a variety of dates. Some was less than one month old, one dose was 11 months old, three doses were three months old, All had been shipped to a veterinarian by reputable manufacturers of biologics and turned over to the author.

The first 20 doses administered were either dead or else of such low virulence that not a single puppy was infected. The 21st caused distemper in the puppy. The 20 uninfected puppies were subsequently given fresh virus made from the spleen of a dog sick with distemper, and all developed the disease.

The dead virus had done nothing toward immunizing them, nor did it cause them to have light cases. The author then wrote to Dr. G. W. Dunkin and learned that in the Laidlaw-Dunkin experiments these men, in using dried virus, depended on it when it had been maintained at —10° F. and when it was not over 10 days old. This is in contrast to the method of shipping stale virus about the country by mail, carrying it in the trunks of automobiles on hot days, or storing it at ordinary refrigerator temperatures, which is the practice today. There is practical evidence that it will live for some time at refrigerator temperatures, but careful data on this point are not available.

Fourteen more puppies have since been injected with dried virus. In each instance the author wrote to the distributors strongly emphasizing the fact that he wished live virus. Of these 14 samples, which came from three manufacturers, seven were potent.

In short, very little dependence could be placed upon the virus part of the Laidlaw-Dunkin method as carried out in field work, using commercial preparations. Is the fact that the virus is so often dead the cause for the breaks in the dogs supposedly immunized with the Laidlaw-Dunkin method? And if it is so difficult to obtain live virus, might this too not be the explanation for such frequent failures in the simultaneous method, which when properly used is exceedingly effective?

Or, if the virus is so often dead, is the vaccine useful without it? We know of dogs apparently successfully immunized without the use of virus at all.

As far back as 1923 Puntoni[1] showed that dogs could be immunized by injections of 10% brain suspensions killed by formalin. Others have confirmed his findings. Lebailly[2] in 1927 showed that spleen suspension will accomplish the same result. Some of his vaccinated dogs showed mild febrile reactions and the rest did not, and yet all appeared to be subsequently immune. Laidlaw and Dunkin[3] gave us the information

[1] Puntoni, V., 1923. *An. d'Ig.*, XXXIII, 558.
[2] Lebailly, C., 1927. *C. R. Acad. des Sci.*, CLXXXV.
[3] Laidlaw, P. P., and G. W. Dunkin, 1928. *Jour. Comp. Path. and Ther.*, XLI, Part 3.

that a 20% spleen suspension, formalized with 0.2% formalin, and followed by an infective dose of live virus, would produce life-long immunity. It was the dosage of live virus, added to the method of their predecessors, which produced the method now in general use, the Laidlaw-Dunkin method.

It has not been emphasized, however, that many of Lebailly's dogs showed no reaction and yet were immune. He used spleens removed from dogs four days after infection. Laidlaw and Dunkin found that this was too early; that spleens removed from their dogs 7 to 16 days after infection produced better results. In the Laidlaw-Dunkin study, a table is presented showing results of immunizing pups with 5 or 10 cc of their vaccine, in which "almost complete protection" developed when live virus was injected only seven days later. It might be argued that seven days was not long enough for complete immunity to have developed. Their field study is reported as follows:

After showing that 340 dogs had been immunized, they stated: "About 50 couple of hounds and 30 dogs have received vaccine made from dog tissue and the remainder vaccine made from ferrets. With a very few exceptions, all hounds and dogs received a final dose of living virus. In nearly every instance the whole procedure went smoothly. One terrier and one foxhound died following, and probably as a result of, the administration of the virus. The degree of resistance called up by the vaccine was variable, for in some instances mild distemper followed injection of living virus. One terrier was acutely ill for a

TABLE I. VACCINATION BY LAIDLAW-DUNKIN METHOD, USING COMMERCIAL PRODUCTS

No.	Breed	Weight (pounds)	Injections (in cc) Vaccine 1/27/37	Vaccine 2/12/37	Virus 2/28/37	Temperatures (minus 100)* 3/3	3/4	3/5	3/6	3/7	3/8	3/9	3/10	Distemper Immune (Exposure 5/12, 5/13)	Recovered	Died
1	Bloodhound	70	5	5	1	2.6	1.8	1.6	1.0	2.0	2.0	2.0			x	
2	Bloodhound	68	5	5	1	2.2	1.6	2.0	1.6	2.8	1.8	1.8			x	
3	Bloodhound	70	5	5	1	2.0	1.8	1.6	2.2	1.4	1.4	1.6				x
4	Bloodhound	52	5	5	1	2.0	2.6	2.2	3.0	3.2	2.8	3.2			x	
5	Bloodhound	52	5	5	1	2.0	2.0	1.4	1.8	3.4	1.2	1.0				x
6	Bloodhound	20	5		1	1.0	1.8	0.6	2.0	2.8	1.2	2.0				x
7	Bloodhound	18	2½	2½	½	0.6	1.8	0.4	1.0	2.6	0.8	1.8				x
8	Bloodhound	21	2½	2½	½	1.0	2.0	0.0	1.0	1.8	0.8	2.2				x
9	Hound	22	2½	2½	½	0.4	2.4	0.2	1.0	1.6	2.0	1.6		x		
12	Hound	34	2½	2½	½	1.6	2.0	4.2	3.4	3.0	2.6	3.0	0.4	x		
13	Bloodhound	20	2½	2½	½	1.6	3.2	3.0	3.0	4.0	2.2	4.3	2.0	x		
16	Bloodhound	74	5	5	1	2.8	2.4	2.2	2.0	3.0	1.6	1.0			x	
19	Bullhound	55	5	5	1	3.2	2.4	1.2	0.8	2.8	1.6	2.4			x	
20	Bullhound	51	2½	2½	1	2.8	2.6	3.2	2.2	3.0	1.6	1.4			x	
21	Bullhound	45	2½		½	3.0	1.6	2.0	1.0	2.0	1.6	2.4				x
22	Bullhound	48	5		½	1.6	1.6	1.6	0.8	2.0	2.0	2.4				x
23	Greyhound	56	5	5	1	3.0	1.4	0.6	1.4	1.4	2.6	2.6		x		
24	Greyhound	44	2½	5	1	2.8	1.8	2.4	1.8	3.2	2.6	2.0	2.4	x		
25	Greyhound	35	5		1	0.6	2.4	1.4	1.6	3.2	3.2	2.4		x		
26	Greyhound	55	5	2½	1	3.2	2.0	1.4	2.0	2.6	1.8	2.6		x		
28	Scottie	15	5	5	½	2.4	2.0	1.6	2.0	3.6	1.6	3.0		x		
29	Scottie	17	2½	2½	1	2.8	2.6	2.0	2.0	4.2	2.0	2.2		x		
30	Beagle	22	5		1	2.4	2.0	1.4	0.6	3.0	1.8	2.0	2.6	x		
31	Beagle	26	5	5	1	2.6	2.0	2.0	4.0	5.0	3.6	3.0	0.4	x		
32	Beagle	21	5		1	2.8	2.2	0.0	0.0	4.2	2.4	2.4		x		
33	Beagle	23	5	5	1	2.4	1.2	0.0	1.0	3.0	1.6	1.2		x		
34	Cocker	14	2½	2½	½	1.6	1.8	2.0	2.0	2.4	1.4	1.4	1.8		x	
35	Cocker	12	2½	2½	½	2.4	3.0	2.2	2.0	3.0	2.8	1.8			x	
36	Cocker	13	5	5	1			3.2	2.0	2.2	2.4			x		
37	Cocker	11	5	5	1			3.4	2.0	2.0	2.0			x		
38	Cocker	11	2½	2½	½	2.0	2.6	1.0	2.0	3.0	2.6				x	
39	Cocker	11	2½	2½	½	2.0	3.2	2.2	2.0	2.6	2.0	2.8	2.6		x	
40	Cocker	11	2½	2½	½	2.0	2.4	2.0	3.0	2.6	2.4	2.4			x	
41	Cocker	11	2½	2½	½	2.0	3.0	1.6	1.8	3.0	2.2	2.4		x		
														16	11	7
					Controls											
10	Hound	21	Untreated			1.6	1.8	0.6	1.0	2.4	1.4	1.0				x
11	Hound	23	Untreated			1.4	1.4	0.6	1.0	2.4	1.8	2.0		x		
14	Bloodhound	17	25 Serum		½	1.0	2.8	0.4	1.2	2.0	1.6	2.2	1.0			x
15	Hound	18	15 Serum			1.0	2.2	0.6	1.2	1.6	1.8	2.2	0.2			x
17	Bloodhound	62	Untreated			3.0	2.0	1.0	2.2	3.2	1.4	1.4			x	
18	Bullhound	40	Untreated			3.2	2.4	0.2	1.0	3.0	2.4	2.0		(Killed)		
27	Greyhound	46	Untreated			2.0	1.4	1.6	3.0	3.2	2.4	3.0				x
42	Cocker	8	Untreated			1.0	2.6	1.4	2.4	2.6	2.2	1.4	1.0			x
														1	1	5

* On March 3, 4, 7, 8, 9 the temperature was taken before feeding; on March 5, 6, 10, it was taken after feeding.

time, but recovered. In most instances no disturbance was noticed after the virus had been given."

Two of their conclusions are as follows: "A dog which has been treated with dead vaccine alone shows resistance to distemper virus, but is not completely immune. . . . A vaccinated dog which has subsequently received living virus becomes immune. The immunity so produced is solid, long lasting, and firm against other strains of distemper virus."

The results of the experiments here reported indicate that these conclusions may require modification.

TABLE II.—VACCINATION ALONE: FRESH VACCINE (31 PUPPIES)
Vaccination: May 8, 1938. Exposure: Dec., 1938

Nos.	Dogs	Appr. Weight (pounds)	Amt. Vaccine (in cc)	Distemper		
				Immune	Recovered	Died
1– 5	Bloodhounds	55	5	2	3	0
6– 8	Greyhounds	50	5	3	0	0
9–11	Bloodhounds	30	5	2	1	0
12–16	Bullhounds	15	3	5	0	0
17	Cocker	22	3	1	0	0
18–20	Beagles	9	3	3	0	0
21–24	Cockers	12	3	4	0	0
25–31	Cockers	8	3	7	0	0
				27	4	0

Commercial Vaccine—Virus Method

Table I shows the treatment and results in vaccinating 34 puppies by the regular Laidlaw-Dunkin method, but using varying amounts of vaccine and virus. Eight controls were placed in the same runs with the treated dogs. Other controls were maintained in separate kennels.

The temperatures are given for eight consecutive days, starting three days after injection of the dried virus. All of the material used in this phase of the study was the product of one manufacturer. Attention is called to the fact that in only a few cases was any reaction noted, if temperature be taken as a criterion of reaction. None of the dogs refused food, and no unusual symptoms were noted. None of the controls contracted the disease from the treated ones, nor did the disease break out among the dogs maintained in separate kennels.

Because there was so little reaction, the author wrote to the representative of the manufacturer and asked for an explanation, for according to Laidlaw and Dunkin some reaction should have been manifested. The reply stated that no reaction occurred because the virus was attenuated, and was not expected to produce a reaction. This did not seem a satisfactory explanation, and the testing of dried virus described above, was commenced. The 21st dose, which produced a violent case of distemper, was obtained from the manufacturer whose representative had written that their virus was attenuated.

Considering the 34 cases in Table I, subsequent exposures have disclosed that 17 were solidly immune, 10 had light cases and recovered, and seven died from distemper. Of the eight controls, one was killed before he had a chance to be exposed, five died of distemper, one had a light case and recovered, one was naturally immune, as were his sire and dam. These were controls mixed with the treated dogs. Among the controls kept in another part of the kennels, the loss was great. Of 30 bloodhound puppies, all died. Of 23 cocker spaniel puppies, 19 died and of six bullhounds, one died. All the dogs used in this study which lived through the first epizootic have passed through two more to date and show solid immunity.

Fresh Vaccine Alone

Another study, frankly accidental, may be used as a comparison for the dogs in Table I and Table III. Thirty-one puppies were vaccinated with spleen suspension, made from the spleen of a large bitch which was killed on the 9th day following artificial infection. It was ground in a sterile mortar with sterile sand, made into a 20% suspension with physiological saline solution, strained and formalized with 0.2% formalin. Then it was stored in a common

refrigerator for 48 hours and injected into the 31 puppies shown in Table II, with the results shown.

There was not enough material to vaccinate any more dogs and so only the choicest ones were vaccinated. The other unprotected puppies in the kennels acted as controls. It had been planned to administer live virus later but this was not done.

Both lots of dogs have been through two epizootics since. The loss among the untreated dogs was 14 out of 16. The treated dogs came through astonishingly well. In comparing them with the dogs in Table I, it seems reasonable to conclude on the basis of these data, that when fresh vaccine of high potency is used, no virus is necessary. The first group was treated with reasonably fresh vaccine, not over two weeks old when the first dose was given, and this was followed by injection of virus (possibly dead) with the result that 17 were solidly immune, 10 contracted distemper and recovered and nine died. In contrast are the dogs in the second group, to which fresh vaccine of high potency was administered, with no virus injection, with the result that 27 were immunized solidly, four contracted the disease and recovered, and none died.

Exposure of the dogs in Table I to natural infection occurred 3½ months after their vaccinations started; exposure of those in Table II occurred six months after their single injection.

A point worth observing is that the amount of vaccine used apparently plays some part in protection, despite the fact that a standard dose for dogs of all sizes is usually recommended. Note in all of the tables presented that where large dogs received the same size dose as the smaller dogs, the former were less well protected. To protect small pups, 3 cc was sufficient; larger dogs appear to require at least 10 cc. By simple proportion we find that if a 10-pound dog requires 3cc, a 70-pound dog should receive 21cc. It is no wonder that if 5cc sometimes protects a 70-pound dog, 3cc almost invariably protects a 10-pound dog.

TABLE III.—VACCINATION ALONE: VACCINE OF UNCERTAIN QUALITY (40 PUPPIES)
Vaccinations: Feb. 10 and 24, 1939.—Exposure: July, 1939

Nos.	Dogs	Appr. Weight (pounds)	Amt. Vaccine (in cc)	Distemper		
				Immune	Recovered	Died
1	Bloodhound	75	10 and 5	0	1	0
2	Dane x bloodhound	70	10 " 5	0	0	1
3– 4	Bloodhounds	50	5 " 5	0	0	2
5–10	Bloodhounds	25	5 " 5	0	3	3
11–13	Foxhounds	16	5 " 5	0	2	1
14–23	Cockers	15	5 " 5	3	5	2
24–26	Scotties	11	5 " 5	3	0	0
27–35	Cockers	11	5 " 5	2	4	3
36–41	Cockers	5	3 " 3	3	2	1
				11	17	13

Does this, then, mean that we would be as well off without virus? Obviously not, because the virus acts as insurance if the vaccine alone does not immunize solidly. When live virus is used on a group of dogs, and a reaction occurs in some of them following its injection, is it not reasonable to conclude that those cases which reacted were those which were not completely immunized by the vaccine? Conversely, when virus is used and no reaction occurs, that is no indication that the virus is dead, for, as we have seen, vaccine alone can immunize dogs. In the latter case we should not expect a reaction, if the interval between vaccination and virus injection is long enough for complete immunity to have developed. (This would seem to be longer than the seven days which Laidlaw and Dunkin waited.)

Older Vaccine Alone

The data in Table III make an interesting comparison with those in Tables I and II. After seeing the results of the treatment of the dogs shown in Table II, the author became persuaded that vaccine alone could be instrumental in producing lifelong immunity. Vaccine was made in Auburn, Ala., placed in a refrigerator for two days, thoroughly chilled and then was shipped to

the kennels at Orange, Conn., where it was again placed in a refrigerator. It was six days old when it was used first, and 20 days old when it was administered as a second injection. It had been unrefrigerated, subjected to the usual vicissitudes of parcel post transport, for three days. The formalin had not been neutralized, as it should have been for longer preservation of the antigenic properties of the vaccine. It was made from two hound dogs, brothers, which were subjected to natural infection, and showed their first temperature rise on the seventh day. Their spleens were removed on the fifth day after the rise in temperature, and prepared in the same way as those mentioned above.

TABLE IV.—COMPARISON OF RESULTS OF TABLES I, II AND III

	Distemper		
	Immune	Recovered	Died
Commercial vaccine, two doses and virus	17	10	9
Fresh home-made vaccine, one dose, no virus	27	4	0
Older home-made vaccine, two doses, no virus	11	17	13

The vaccine was injected into dogs, as shown in Table III, Feb. 10, and Feb. 24, 1939. In July, five months later, the puppies were subjected to an epizootic of distemper. At the time of injection all the puppies in the kennel over two months old were injected. But before the epizootic broke out many puppies had been born, including several litters of bloodhounds, one large litter of bullhounds, and several litters of cocker spaniels. Some puppies from each group had been sold. The loss among the unvaccinated bloodhounds was 17 out of 19; of the bullhounds only two out of seven died; of the cocker spaniels, 12 of 16. The bullhounds, the result of crossing bull terriers with bloodhounds and inbreeding, constitute a very hardy breed, and should probably not be included in these results, but even including them, the total loss was 31 out of 40, or 77%. (These puppies were not treated with serum.)

In contrast to the heavy loss among the unprotected dogs, the vaccinated ones showed 11 immune, 17 contracting distemper and recovering, and 13 dying—a loss of 31.8%. Vaccination would seem to have been eminently worth while in this case, even where live virus was not used. Comparing the results in Table I, where dried virus was used, with those in Table III, where no virus was used: in the former, nine died out of a total of 36; in the latter 13 died out of a total of 41. The comparison is 25% against 31.8%; but the first exposure came at 3½ months in the case of dogs in Table I, and at five months for the dogs in Table III.

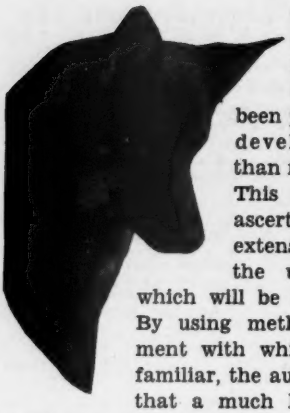
TABLE V.—COMPARISON OF RESULTS OF ALL METHODS WITH CONTROLS

	Distemper		
	Immune	Recovered	Died
Vaccinated dogs, all three methods	55	31	22
Controls, untreated	1	43	104

Table IV compares the three methods used. It is not claimed that fresh vaccine will always achieve such results without virus. but it is obvious that sometimes such results can be obtained, and it is apparent that here we have at least one explanation of the efficacy of the Laidlaw-Dunkin, or Lebailly, treatment, even when the dried virus is dead. (The use of vaccine alone should, in all justice, be called the *Lebailly method.*)

Probably with the greater care now being given to the distribution of fresh, refrigerated distemper biologics, much better results can be expected than those shown in Tables I and III. But even with such a poor showing, the combined mortality was only 22 out of 108, or 20%. In the untreated controls the mortality was 104 out of 148, or 70%, a difference greatly in favor of immunization even by imperfect methods (see Table V).

A 70% mortality is above the general average for distemper. This may be partly accounted for by the fact that the dogs were allowed to continue, for the most part, in the same pens in which they had been living, and recover or die without treatment. Again, many were bloodhounds of exaggerated type—a breed of low resistance to the disease. Some outstanding specimens were taken inside, and an attempt was made to treat them, usually to no avail. While no accurate check was kept, it is estimated that about as many controls as vaccinated dogs were treated. With serum at hand, many of



the dogs which died might have been saved, or even been prevented from developing more than mild symptoms. This was definitely ascertained by an extensive study on the use of serum which will be reported later. By using methods of treatment with which he is now familiar, the author feels sure that a much larger number of the sick dogs might have been cured.

The author wishes to emphasize that the disease with which he dealt was genuine distemper with all the usual symptoms, including characteristic temperature curves, secondary invaders and long drawn course. Years of handling hundreds of cases, and living with sick dogs, almost rule out the possibility that such a large number of cases could be mistaken for one of the diseases which do at times, in small numbers, cause confusion. Moreover, the fact that all three groups of dogs, or a large part of each group, has been repeatedly subjected to the same disease, and that all of the conclusions are drawn on this basis, rules out certain criticism which could be justly urged had conditions been otherwise. The number of dogs represented by these data is quite considerable, and in fact a much larger number than those used in the Laidlaw-Dunkin experiments. As mentioned in a previous study[4] the need is again emphasized for uniform genetic stock in such experiments as these.

Conclusions

1. It is possible to immunize a dog solidly by the use of high potency fresh vaccine alone. This fact probably accounts for those cases in practice where no reaction follows the use of live virus.

2. Commercial dried virus, unless quickly handled and maintained at low temperatures, may be dead at the time of injection.

3. Virus is to be regarded as insurance, in the event that low potency vaccine is used which does not completely immunize but which does give some protection.

4. The cause of breaks in vaccination lies sometimes with the vaccine, which may not be sufficiently fresh or of sufficiently high potency, and sometimes with dead virus.

5. It is advisable to grade the dose of vaccine to the size of the dog, rather than to administer a standard dose to dogs of all sizes.

6. Even with imperfect methods, vaccination is worthwhile, this study showing a mortality in vaccinated dogs of 20% against 70% for the unvaccinated controls; both percentages being higher than would be the case normally because little or no treatment was given after the dogs became sick.

[4] Dickerson, V. C., and L. F. Whitney, 1938. *Jour. Exp. Biol. & Med.*, 38, 263.

* * * *

Left: Dr. Jas. E. Green, Director of the Small Animal Clinic in the Veterinary School at Auburn, Alabama. Right: The author when a student at Auburn. Doctor Whitney now has a son in the Alabama school. The apparatus which Drs. Green and Whitney are holding is a simple and convenient appliance for giving an enema to a dog. This photograph was taken at the 16th Annual Conference for Graduate Veterinarians, Alabama Polytechnic Institute, Auburn, Ala.

The Experimental Use of Phosphoric Acid Intravenously in Dogs

By B. J. ELANDER,*
San Diego, California

IN the course of research conducted by the author and R. de R. Barondes, M. D., F.R.S.A.,F.F.Sc., on alkalosis, arteriosclerosis and other abnormal acid-base states, the intravenous use of phosphoric acid was suggested. Phosphoric acid was chosen because a resultant stabilized calcium-phosphorus ratio was desired.

Calcification is known to play an important role in arteriosclerosis, but the factors determining the deposition of the lime salts in the tissues are still undetermined. There are a number of divergent theories advanced to explain this calcification. Medial calcification occurs chiefly in the walls of the large arteries of the extremities. Some authorities believe that it begins in apparently normal muscle fibers and extends in circular and longitudinal directions to form calcified rings; others contend that the calcification process begins in the minute ramifying vessels (vasa vasorum) within the walls of the arteries regardless of muscular tissue. The later trend of thought is that these arterioles become so obstructed that anoxemia and degenerative changes of the arterial walls occur, thereby increasing the work of the heart to force the circulation through these affected areas, with a resulting arteriopressor.

Our experiments have shown that arteriosclerosis in dogs is more common than is generally believed. This is particularly true in old, fat house dogs fed heavy alkaline diets with restricted exercise. Dogs getting a sufficient amount of exercise and plenty of meat in the diet seem to be quite free from this condition except where some infection has predisposed the animal to it. In the excessive feeding of parathyroid extract, cholesterol, and irradiated ergosterol, and in hypervitaminosis D, we find an organic disturbance which excites a metastatic calcification (which is a translocation of the calcium from the skeleton to the soft tissues.) The salts of calcium deposited in the arteries and soft tissues are the same as those found in bone. There is also a small percentage of calcium fluoride. This varies in proportion to the geographical location; the quantity is small in direct ratio to the other constituents of bone, but is essential as a catalyzer, acting to sensitize the phosphates, thus enabling the phosphorus and calcium to combine with the tissues.

All processes of metabolism and nutrition depend upon the circulatory system; therefore, in order to have the proper mineral ratio in the body, it is essential that a normal blood picture be present. The stability of the blood is protected by three lines of defense: first, the buffer system,

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maintaining the alkalinity of the blood between pH 7.2 and pH 7.4, notwithstanding the carbonic, phosphoric, sulphuric, lactic and other organic acids which are constantly being formed in the process of metabolism; second, the excretion of CO_2, and third, the excretion of fixed acids.

Carbonic acid is a product of tissue activity and is produced in greater quantities than any other acid. It is buffered mainly by a base released from hemoglobin. When carbon dioxide enters the blood the sodium bicarbonate of the plasma increases to offset the lowering of the pH, and as the blood passes from the arterial to the venous side, CO_2 is absorbed and diffused into the red blood cells. Here, through the action of an enzyme, carbonic anhydrase, it is converted to carbonic acid (H_2CO_3). Under abnormal conditions, this enzyme may become inactive or function improperly, and the carbon dioxide is not converted, resulting in a bicarbonate increase. This raises the pH of the blood and an alkalosis is present, which in turn disturbs the calcium content of the blood stream because the calcium surplus is in an unassimilable form, resulting in an uncompensated alkali excess. In other words, the sodium bicarbonate is increased without a proportional rise in carbonic acid; this may result from pathological changes in the blood stream as well as from the ingestion of large quantities of alkali, or from the loss of acids in dehydration and vomiting. When an excess of alkalis remains in the blood stream, with a resulting high pH, there can be no compatibility with respect to the calcium:phosphorus ratio of the blood stream. As a result, the calcium and its carbonates, etc., do not remain in solution but are deposited in the surrounding tissues as free alkalis.

The intravenous injection of the phosphoric acid solution (1:600) with a magnesium ion buffer was adopted to lower the pH in animals which were on a heavy alkaline diet and exhibitng some degree of circulatory distress. Phosphoric acid was used not only to lower the alkalinity of the blood stream but also to balance the calcium-phosphorus ratio so that the calcium would be soluble and easily assimilated in its proper ratio throughout the body.

All dogs chosen for the experiment were over seven years old, and the majority were suffering from senile obesity. Small test doses were given at first to determine the animal's tolerance to the acid. The animals showing no systemic reactions to the test injections, the dosages were increased daily until the saturation point was reached. The starting dosage of 5cc was increased by 1cc daily until 25cc doses were being given. These were continued daily for a period of three weeks to see if phosphorus excess in the calcium:phosphorus ratio would occur to a degree where there would be an absorption of calcium with a resultant softening of the bones. Over a period of time with various higher concentrations there was no evidence of osteomalacia.

Results

In general, the body metabolism was accelerated and the animals had more vigor as a result of the phosphoric-acid injections. The blood pressure in all cases was lowered and there were no systemic changes with small doses from 5cc to 10cc. In most of these animals the blood pressure had been abnormal, varying from 160 to 172 systolic and from 96 to 100 diastolic. After the injections were given the pressure dropped to between 120 and 145 systolic and 82 and 94 diastolic. This was not merely a temporary drop; the pressure remained down over a period of time, in some cases for as long as three months after the injections were discontinued.

Other changes noticed in the behavior of the animals were increased sexual desire in the males, and, in aged obese dogs, relief from discomfort in hot weather. Whether this was a result of the change of the blood chemistry from a high alkaline to a low acid reaction, or of the lowering of the arterial pressure by normalizing the calcium output from an unbalanced calcium: phosphorus ratio, I cannot say. During the early course of the experiment the appetite was improved greatly, but after saturated doses had been continued for some time a slight inappetence was observed.

An Intestinal Protozoon of Dogs*

Giardia canis was classified by Hegner in 1922. Tsuchyya later proved that there are two distinct strains of this organism. It is a protozoon found in the intestines of dogs; Hegner has found it in cats also. The trophozoites measure 13.8 microns in length and 8.5μ in breadth. The cysts have a mean length of 11.1μ and a mean breadth of 8.2μ. The habitat is the upper intestine, where the trophozoites are found in large numbers adhering to the lining epithelium by means of a sucking disc.

The chief symptoms noticed in *G. canis* infestation are loss of appetite and a recurrent diarrhea. According to Chandler, the anorexia is due to the large numbers of protozoa covering the intestinal epithelium, preventing proper absorption; this also causes the animal to have a distressed appearance. Breuer found that common symptoms are gastritis, duodenal ulcers, hemolytic icterus, and subnormal temperature. Blood and pus may be found in the feces, and defecation may be accompanied by tenesmus. When of long standing the condition results in anemia and slow growth. Pain, when present, is usually in the right upper quadrant, although it can extend over the entire abdomen.

Hegner found that a carbohydrate diet is more favorable to an increase in the number of flagellates than a high protein diet.

Chandler estimated that 300,000,000 cysts are voided in a single defecation. His technique for demonstrating the cysts is as follows: place a specimen in the centrifuge tube, mix with plain water, and centrifuge as for hook worms, three minutes at 1500 to 2000 r. p. m.; decant the fluid and add a zinc sulphate solution that tests 1,180 with a hydrometer centrifuge. Allow this to stand a short time, then loop off the surface film, place a cover slide over it, and examine under low power; use Lugol's solution to color for examination under high power.

Treatment with atabrine, a basic acridine dye, will cure the majority of cases. Administer a 1½ grain tablet morning, noon and night for five days. Usually there are no more cysts in the feces after three days of this treatment, but it is advisable to continue it for the additional two days. The dosage mentioned is for dogs weighing 20 pounds or over; smaller doses might be advisable for lighter dogs and cats. McKee noticed a Dobermann that reacted unfavorably to atabrine, howling for some time after treatment. Such reactions should be watched for, particularly in nervous animals.

Houston, Tex. J. GILBERT HORNING.

* Presented at the 16th Annual Conference for Graduate Veterinarians, Alabama Polytechnic Institute, Auburn, Ala., March 20-22, 1940.

SMALL ANIMAL HOSPITAL, SCHOOL OF VETERINARY MEDICINE, MIDDLESEX UNIVERSITY
This recently-completed small animal hospital will be dedicated this fall, when the third year of Middlesex University's veterinary course commences. Construction on the main veterinary building, a three-story structure, which includes the large animal hospital, is still in progress; it is expected that it will be completed by September. Meanwhile, the building here shown is being used as a temporary anatomical laboratory.

Abstracts

Control of Brucella Infection

Rolle[32] describes the eradication of *Br. abortus* infection from a herd in the course of five years by isolating the new-born calves and young stock from the affected animals, and inoculating the young stock with non-virulent live vaccine before returning them to the infected herd. A further precaution was the immediate slaughter of all animals found to be actively excreting the organisms in their milk and discharges. He urges that all suspected cases of contagious abortion in cattle should be made notifiable to the competent authorities.

* * * *

Salt and Brine Poisoning

After observing accidental cases of brine poisoning in pigs, Wautié[36] fed 675gm (22½ oz.) of pure NaCl to two pigs, each weighing 35kg, (77 lbs.) over a period of 34 days. Mild diarrhea was the only symptom noted, and he concluded that it was difficult to poison pigs with salt since they refuse food containing it in sufficient quantity. In further experiments 100gm of salt were ingested in 24 hours when the pigs had been previously starved, and in others 100 to 150gm were given in one to 1½ litres of water by stomach tube. No serious disturbance followed, but 150gm by stomach tube caused vomiting. A low blood calcium was noted in one case, but was normal in two days. In all cases the pigs weighed 30kg (66 lbs.).

In three fatal cases of brine poisoning, two died in convulsions within two hours, but of two experimental pigs each receiving 950cc over a period of eight days, one died of acute enteritis and ascites, and the other recovered. Wautié confirms the confusion that exists as to the toxic dose and symptoms of salt and brine poisoning. As to salt poisoning, he thinks the Na cation responsible rather than the Cl anion or the whole molecule, but does not explain its action. He does not recommend the use of saline purgatives with a Na base in animals with a nervous or humoral tendency. Low blood calcium (he found 0.146mg per cent to be normal for young pigs) is frequently found, but tetanic symptoms may appear when the calcium level is normal. He considers the injection of calcium to be the best line of treatment.

* * * *

Dogs and Cats as Sources of Tuberculosis in Man

Hjärre[37] gives an account of cases of tuberculosis in 175 dogs and 22 cats, all of which were observed at general routine post-mortem examinations at the Stockholm Veterinary College between 1908 and 1938. During the period there was a steady increase and in the last decade about 5% of the dogs and cats were found to be tuberculous. The findings are analyzed with reference to age, sex, portal of infection and pathogenesis, and the type of bacillus responsible for the infection.

In dogs over one year of age, males were affected three times as often as females, and the same applied roughly to the cats. In dogs the infection was mostly *via* the respiratory tract, whilst in cats it was mostly peroral. The type of bacillus was investigated in 74 dogs and seven cats. The human type was present in 57 (70%) of the dogs and in one cat, the bovine type occurring in the rest. The internal organs most frequently affected in dogs were the lungs, liver, pleura, kidneys, peritoneum, pericardium, spleen, myocardium, and intestine (in descending order). In cats the corresponding order was lungs, liver, pleura, kidneys, spleen, peritoneum and myocardium.

Herlitz[38] observed the members of families in whose houses a tuberculous dog or cat had lived, and sent a questionnaire on this subject to other veterinary practitioners in Sweden. Investigations were made on

[32] Rolle, M., 1938. Ueber die Bekämpfung der Brucellose. III Balti Valsty. vet Kongr. Protocol., pp. 48-50. Abst. in the *Vet. Bul.* 10:3, p. 164.

[36] Wautié, M., 1939. Contribution à l'étude de l'intoxication par les sels de sodium and les saumures. *Ann. Méd. Vét.* 84, pp. 349-369. Abst. by R. Bamford in the *Vet. Bul.* 10:5, p. 380.

[37] Hjärre, A., 1939. Ansteckungsquelle für den Menschen über Tuberkulose bei Hunden und Katzen. *Acta Tuberc. Scand.* 13, pp. 103-124. Abst. in the *Vet. Bul.* 10:5, p. 319.

[38] Herlitz, C. W., 1939. Die Uebertragung der Tuberkulose zwischen Menschen und gewissen Haustieren, besonders Hunden und Katzen. *Acta Tuberc. Scand.* 13, pp. 125-162. Abst. in the *Vet. Bul.* 10:5, p. 320.

families who had owned 65 tuberculous dogs and three tuberculous cats. In the case of 52 of these dogs and one cat there was no tuberculosis in the family, although in 20 of the dogs there had been open tuberculosis and the animals had been in close contact with children. Fifteen case records of tuberculosis in persons are given; dogs were concerned in 13 of them and cats in two, all the animals having open tuberculosis. Eight of these dogs had human type infection and had presumably been infected by their owners; one had bovine type tuberculosis. There were no data for the remainder. One of the two cats had a bovine type infection and in the other the type was not determined. Evidence that infection had passed from animal to man was judged probable in two cases only.

In reply to the questionnaire, 22 practitioners reported a total of 35 cases of tuberculosis in dogs and three in cats. Eleven of the dogs had lived with tuberculous families, and in one case infection was held to have passed from dog to man, in five cases from man to dog, and in the other five cases infection might have passed in either direction.

In general the inquiry suggested that roughly one-third of all cases of tuberculosis in dogs and cats originate from tuberculous owners, and that the danger of tuberculous dogs and cats infecting their owners is not as great as might be supposed.

Book Reviews

The Nutritional Requirements of Dogs. *By Clive M. McCay,* Ph.D., Professor of Animal Nutrition, Cornell University. 32-page pamphlet, published by the author, Ithaca, N. Y., 1939.

This pamphlet constitutes a very useful summation of the scientific information available on canine nutrition down to the date of publication. The results of some important research conducted last year are not included, but that is still readily available to most veterinarians in their veterinary periodicals of last year and this. Nearly four pages of bibliography are given.

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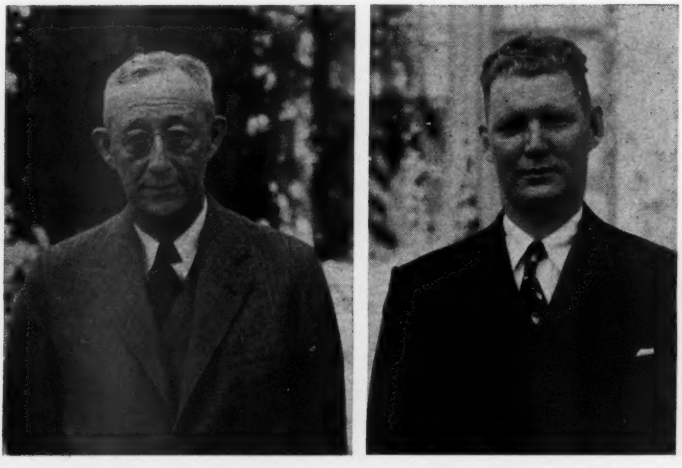
Poultry Practice: *A Collection of Discussions on Poultry Diseases and Related Subjects.* By L. D. Bushnell, E. J. Frick, M. J. Twiehaus and others. Cloth bound; 160 pages, 81 illustrations. Veterinary Magazine Corp., Chicago, 1940. $1.00.

About this little book the *Veterinary Journal* [London] says:

Excellent descriptions are given of the various diseases of poultry as they occur in America, and in many instances the various lesions are illustrated with such detail that they form a good indication for recognition of the disease condition as it occurs in the affected fowls. Though descriptions of diseases and their control form the bulk of the book, due prominence is also given to poultry husbandry, e.g., chapters deal with such subjects as incubator hygiene, poultry flock sanitation, etc. This is all to the good, for it is being clearly realized that in the control of



Leland D. Bushnell (left) and Edwin J. Frick, who collaborated in the preparation of *Poultry Practice*.

diseases of animals, and this is no less true of poultry, their husbandry must also be the subject of study and practice.

The value of this book lies in its conciseness, there being gathered together such a wealth of information in a limited space to which so many well-known authorities have contributed.

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The Principles of Parasite Control. *By Thomas W. M. Cameron,* T.D., M.A., B.Sc. (Vet. Sc.), Ph.D., D.Sc., M.R.C.V.S., F.R.S.C., Director, Institute of Parasitology, Professor of Parasitology, McGill University, Macdonald College, Quebec, Canada. Cloth bound; 48 pages. National Business Publications, Limited, Gardenvale, Quebec, Canada, 1940. 50c.

This little volume is essentially a series of three lectures delivered at the Royal Veterinary College [London] in September, 1938. It constitutes a highly satisfactory treatise of the subject indicated in the title. The discussion includes a consideration of the following subdivisions of the subject of parasite control: Parasitism; Importance of parasites; The parasitic environment; Concepts of immunity; Control of parasites; Methods of destroying the egg or pre-infective larva; Manure treatment; Strongyle life cycle; Manure distribution; Larvae not in manure; Prevention of trichinosis; Methods of destroying infective larvae; Avoidance of ingress; Vectors; Destruction within the host; Reservoir hosts; Avoidance of parasitic disease.

A single paragraph from the discussion of the importance of diet in the prevention of parasitic disease indicates how fully up-to-date the treatise is:

> It is extremely difficult to separate many parasitic diseases from those caused by deficiencies in diet. There is evidence to suggest that in many cases the two are so closely interwoven as to be inseparable and that their effects are mutually cumulative. There is no doubt that in very many cases, a sufficient, well balanced diet, balanced in all its accessory factors as well as its main constituents—will prevent parasitic disease and will often actually reduce the numbers of parasites harbored. Pasture treatment, such as "top-dressing," is often a valuable way of doing this. This adequate diet is particularly important for immature animals and every effort should be made to secure this. This is, of course, true for all preventive measures to be taken; it is especially important in connection with diet.

The Milking Goat. *By M. R. Steffen,* V.S., M.D.C. Paper bound. 69 pages. Will L. TeWalt, Vincennes, Ind., 1940. Price $1.00.

This little book was written for two purposes: 1. To put the case of the goat as a producer of milk on a sound basis. 2. To help the beginner in goat keeping.

Mart Steffen will be remembered as the author of five veterinary texts published 20 to 25 years ago, and as a frequent contributor to the veterinary periodical literature of that period. This is an animal husbandry discussion—in no sense a veterinary text. It is written in the well-known direct and forceful manner of the author, who is seldom perturbed by doubts; a thing either is or isn't; it's black or white; all good or all bad to Steffen. He doesn't use "weasel words."

Veterinarians whose experience has included little contact with the milking goat will find this a clear, concise, informative and useful little volume. Unfortunately the publisher's price is not in keeping with the appearance of the volume; the contents however justify the cost.

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The Science and the Mystery of the Cat. *By Ida M. Mellen,* Honorary Vice-President of the Allied Cat Lovers, Inc. Cloth bound. 274 pages; 40 illustrations (chiefly photographs). Charles Scribner's Sons, New York, 1940. $2.75.

Using the terms science and mystery to mean facts and fancies, the author has deemed no subject pertaining to cats foreign to the discussion. In the foreword she tells us that the period of her study of cats has been limited to two dozen years, but that the volume contains more than 100 new facts and entire chapters on aspects of the cat not heretofore noticed.

It will indeed require a thoroughgoing felinophile to be interested in all parts of this volume, which is long on the fanciful; there are such persons, however. For a far larger number, the bringing together in a single volume of material so wide in scope and varied in detail will be a convenience. It should prove a satisfactory volume for the veterinarian's waiting-room.